RUMPHIUS PATTIMURA BIOLOGICAL JOURNAL
Vol 1, No 1, 2019, pp 008– 011
E-ISSN : xxxx - xxxx

THE STUDY OF ADMINISTRATION EXTRACT KEBAR'S GRASS ON NUMBER LEYDIG CELLS AND SERTOLI CELLS IN RAT *Rattus norvegicus* THAT EXPOSED WITH CIGARETTE SMOKE

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ABSTRAK

This research aimed to study on the microscopic changes of number leydig cells and sertoli cells. A factorial CRD with periode of treatment and sample collection was applied in this study. An exposure of cigarette smoke was carried out at 10 cigarettes/rat/day for 2.5 hours in a smoking chamber. Extract kebar's grass given was 0.0945 mg/g body weight/day. Twenty seven (27) of male rats were divided into four groups. N group was untreated animals, 20h group is group that was expose to cigarette smoke for 20 days then given administration extract kebar's grass for 20 days and stopping treatment for 20 days and 60h group is group that was expose to cigarette smoke for 60 days then given administration extract kebar's grass 60 days and stopping treatment for 60 days. Data collection was carried out at twice that after cigarette smoke exposure (T0), second after administration extract kebar's grass (T1) and third after stopping treatment (T2). The parameters measured were the number of leydig cells and sertoli cells. That increasing number leydig cells and sertoli cells of rat exposed to cigarette smoke after giving seaweed extract kebar's grass for 20 days and 60 days

Kata Kunci: Cigarette smoke, Kebar grass, Leydig cells, Sertoli cells

INTRODUCTION

Cigarette smoke is a heterogeneous aerosols from tobacco burning. Each cigarrete contains various chemicals such are acreolin, carbon monoxide (CO), nicotine, ammonia, formic acid, hydrogen cyanide, nitrogen oxide (NO), cyanogen, phenol, acetone, methanol, and tar [1]. Chemical content of tobacco that has been identified amount 2,500 components, whereas in cigarette smoke has been identified as many as 4,800 kinds of chemical components that can be harmful to health including tar, nicotine, CO, and NO. Cigarette smoke contains free radicals in very high number, estimated in one puff of a cigarette there are 1,014 molecules of free radicals [2]. The most dangerous free radicals contained in cigarette smoke is CO which can cause damage to the cell membrane. Cigarette smoke can cause structural changes in the respiratory and decreased immune response [3]. Changes in the structure and cell membrane damage can occur to all cells, including cells of leydig and sertoli cells in the testis because free radicals carried in the blood to the organs of the testes. This phenomenon can be repaired with traditional herbal therapies such as kebar's grass originating from Papua Arfak mountains, especially the District kebar

Kebar's grass contains substances such as flavonoids, vitamin E and vitamin A is an antioxidant that can neutralize toxic, toxic prevent damage and maintain healthy spermatozoa. Vitamin E can inhibit oxidation reactions by binding to vitamin E to vitamin E free radicals that function again as antioxidants [4]. The presence of active agents such as agents antioxidants, nutrients and amino acids contained in the grass kebar entering through the blood to all the cells and tissues of the body, including the testis, is expected to improve testicular function so as to improve the leydig cells and sertoli cells. This research aimed to study on the number of leydig cells and sertoli cells of male rats exposed to cigarette smoke after giving extract of kebar's grass

METHODS

The materials used in this study is adult male rats Rattus norvegicus galur Sprague-Dawley as many as twenty four rats, 12 weeks old and weighing 150 grams were obtained from the Animal Facility, Faculty of Veterinary Science, Bogor Agricultural University. Other materials used is clove cigarette and 5 kg of kebar's grass taken from Kebar District, Manokwari, West Papua. The tools used in this study are the smoking chamber and microscope. Making extract of kebar's grass done using all parts of the kebar's grass ie roots, stems and leaves that have been dried by the drying sun as much as 5 kg made into extracts of kebar's grass in the laboratory Balitro. Determination of the dose of seaweed extract kebar's grass in rat is 0.0945 mg/g body weight/day were obtained from the conversion of the administered dose in mice is 0135 mg/g body weight/day [5] based on the conversion of the dose according to Reference [6]

The rats placed in cages of platic box covered with wire and husk as the base. Feed formed pellet (PT. Japfa Comfeed Indonesia) and water were given ad libitum. Environment of cage is made to avoid damp, adequate ventilation with lighting for 14 hours and darkness for 10 hours. Each rats was placed in cages per treatment group. Before treatment, the animals were adapted to the atmosphere of the cage for one week. Twenty seven (27) of male rats were divided into four groups. N group was untreated animals, 20h group is group that was expose to cigarette smoke for 20 days then given administration extract kebar's grass for 20 days and stopping treatment for 20 days, and 60h group is group that was expose to cigarette smoke for 60 days then given administration extract kebar's grass 60 days and stopping treatment for 60 days. Data collection was carried out at twice that after cigarette smoke exposure (T0), second after administration extract kebar's grass (T1) and third after stopping treatment (T2). Exposure to cigarette smoke was held on the mornings and afternoons. The extract kebar's grass held in the morning. The parameters measured were the number of leydig cells and sertoli cells.

This study uses a Completely Randomized Design Factorial with treatment time and retrieval time factors. The results were analyzed by Analysis of Variance (ANOVA) follow Duncan Test at significance level $\alpha = 0.05$ using SAS sofware.

RESULT AND DISCUSSION

The average leydig cells and sertoli cells after exposed with cigarette smoke, continued with giving of extract kebar's grass and stopping treatment the group 20 days and 60 days are presented in Table 1. Statistical analysis showed that there was an interaction between duration of exposure to cigarette smoke, duration of long extract kebar's grass and dismissal treatment of the number of leydig cells and sertoli cells ($P<0.05$). Long exposure to cigarette smoke significantly ($P<0.05$) to decreasing number of leydig cells and sertoli cells. After administration extract of kebar's grass significant effect to increasing number leydig cells and sertoli cells on all the groups of rats that had been exposed by cigarette smoke and after stopping treatment, number leydig cells and sertoli cells continued to show improvement ($P<0.05$). The group given extract kebar's grass for 60 days to have number of leydig cells and sertoli cells more than 20 days.

Long exposure to cigarette smoke significantly ($P<0.05$) to decreasing number of leydig cells and sertoli cells to group 20 days and 60 days compare to control group. Statistical analysis showed that there are significant differences ($P <0.05$) between control and treatment groups. This is thought to occur due to the inclusion of toxic substances from the cigarette smoke coming through the endocrine glands testicular lead to degeneration of leydig cells and sertoli cells. This is consistent with several studies on the effects of the compounds contained in cigarette smoke that has negative effects such as inhibiting spermatogenesis, damaging even to cause the death of cells in the reproductive organs. Giving PAH compounds can becaused atrophy of the seminiferous tubules, damage of sperm morphology, decrease of testosterone and LH [7]. nicotine exposure can decrease number of leydig cells [8] and administration by timbal of lead cause DNA damage spermatogenesis and Sertoli cells in mice [9]. According to reference [10], inhaling cigarette smoke either actively or passively, and then will be followed by absorption of substances in the smoke by the blood vessels in the lungs and circulate in the blood circulation and allows the deposition of toxic substances such in seminal plasma through various means such as diffusion and active transfor, eventually these substances induce cell death as happened in leydig cells and sertoli cells.

Long administration extract Kebar's grass in rat that had been exposed to cigarette smoke showed an increase in the number of Leydig cells and Sertoli cells. This phenomenon explains that the longer granted extract kebar's grass can increase the number of Leydig cells and Sertoli cells of rat exposed to cigarette smoke and even after stopping the extract kebar's grass showed an increase in the number of Leydig cells and Sertoli cells. Kebar's grass containing flavonoids, vitamin A and vitamin E acts as an antioxidant to scavenge free radicals from cigarette smoke and stimulate the regeneration of Leydig cells and Sertoli cells of the testis. Kebar's grass working mechanisms in the testes in enhancing the activity of cells that produce

FSH and LH in the pituitary. Power spontaneous and rapid stimulation of the hypothalamus causes a signal to increase the secretion of folicle Stimulating Hormone (FSH) and luteinizing hormone (LH).

Table 1. The average number of Leydig cells and Sertoli cells of rats after exposure to cigarette smoke, giving extract kebar's grass and stopping treatment.

Parameter	Exposure Time	Taking Date Time			Average
		T0	T1	T2	
Leydig Cells	N	31.00±0.02[a]	30.67±0.02[a]	31.33±0.01[a]	31.00
	20h	27.00±0.10[c]	32,33±0.29[b]	35.33±0.12[a]	31.55
	60h	25.67±0.01[c]	35.00±0.02[b]	44.00±0.05[a]	34.89
	Average	27.89[ac]	32.67[ab]	36.88[a]	P<0.05
Sertoli Cells	N	26.00±0.02[a]	26.66±0.01[a]	26.67±0.02[a]	26.45
	20h	24.33±0.78[b]	24.67±0.87[b]	28.67±0.39[a]	25.89
	60h	20.67±0.93[c]	37.33±0.29[b]	58.66±0.63[a]	38.89
	Average	23.67[bc]	29.56[ab]	38.00[a]	P<0.05

Note: Data shown are mean values ± standard deviation. Different letters in the same column indicate significant difference (P<0.05). N group was untreated animals, 20h group is group that was expose to cigarette smoke for 20 days then given administration extractkebar's grass for 20 days and stoppingtreatment for 20 days, and 60h group is group that was expose to cigarette smoke for 60 days then given administration extractkebar's grass 60 days and stopping treatment for 60 days. T0 = after cigarette smoke exposure, T1 = after administration extractkebar's grassand T2 = after stopping treatment.

Reproductive physiology center located in the hypothalamus and pituitary to control the production of testosterone and spermatogenesis in males. Ties and activation of a specific receptor that functions to regulate gonadal function and reproductive hormone secretion in the hypothalamus results in increased secretion of Gonado Releasing Hormone (GnRH) and flowed into the anterior pituitary gland. Gonado Releasing Hormone (GnRH) is secreted and then stimulate the anterior pituitary gland secretes and releases a number of gonadotropic hormones, namely Luteinizing Hormone (LH) and folicle Stimulating Hormone (FSH). Effect of increased secretion Gonado Releasing Hormone (GnRH) causes increased secretion of luteinizing hormone (LH) and folicle Stimulating Hormone (FSH) from the anterior pituitary gland. Setting gonadal function and stimulate the production of steroid sex occurs through a bond between Luteinizing Hormone (LH) and Luteinizing Hormone Receptor (LH-R) for the proliferation and development of Leydig cells and folicle Stimulating Hormone (FSH) with folicle Stimulating Hormone-Receptor (FSH-R) for the proliferation and development of Sertoli cells in the testis [11].

Increased secretion of luteinizing hormone (LH) affect the increase in the number of Leydig cells produced. According to reference [12], Leydig cells produce testosterone in reproductive function that plays a role in spermatogenesis cycle and induce lust in males. Increased production of testosterone which is an important factor in the cycle of spermatogenesis and increased lust after giving extract kebar's grass causing a longer period of lust in males. Increased secretion folicle Stimulating Hormone (FSH), which is believed to be due to post extract kebar's grass also increases the number of Sertoli cells through proliferation. According to reference [13], the folicle Stimulating Hormone (FSH) secreted anterior pituitary involved in producing Sertoli cells which produce Androgen Binding Protein (ABP). Sources of nutrients in the process of maturation of sperm cells derived from Androgen Binding Protein (ABP) which is produced by the Sertoli cells [14]. Testosterone and Sertoli cells which produce Androgen Binding Protein (ABP) in large quantities used in spermatogenesis cycle to increase the number of sperm cells. The number of Sertoli cells increased after the administration of seaweed extract kebar's affect production increase Androgen Binding Protein (ABP) resulted in the availability of nutrients in large quantities in the process of maturation of spermatozoa.

CONCLUSION

Administration of extract kebar's grass for 20 days and 60 days can increase the number of Leydig cells and Sertoli cells of male rats that had been exposed to smoke.

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