Role of Lingzhi mushroom (Ganoderma lucidum) polysaccharide peptide (ß-glucan) on sexual behaviour, testicle testosterone testis and mitotic index of old male Brown rat (Rattus norvegicus)

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Full Length Research Paper

Role of Lingzhi mushroom *(Ganoderma lucidum)* polysaccharide peptide (ß-glucan) on sexual behaviour, testicle testosterone testis and mitotic index of old male Brown rat (*Rattus norvegicus)*

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Abstract

Late Onset Hypogonadism (LOH) is a syndrome characterized with decline in physical ability, sexual and psychological ability associated with decreased of testosterone in the blood. In middle age, around 45 – 59 years of age, reproductive function and hormones such as testosterone started decline. In accordance with the increasing age, the testosterone production also decreased, which is known as the aging process. The increase of life expectancy also increases the number of elderly in the future. Thus, the elderly problems also increased. *Ganoderma lucidum* polysaccharide peptide which is known as ß-Glucan has been used as traditional medicine in China and Japan for hepatitis, hypertension, chronic bronchitis, asthma bronchial, cancer and some other conditions. This study aimed to analyze the effect of β Glucan extract from *Ganoderma Lucidum* Polysaccharide Peptide (PSP) for 21days compared to β Glucan ethanol extract of *Ganoderma lucidum* Polysaccharide Peptide (PSP) on sexual behavior, testosterone, and mitosis index of old male (*Rattus norvegicus)* rat testis. To determine male *R norvegicus* sexual activity, it used CCTV incaged inside the cage made of acrylic for 21days. Testicular testosterone was examined using immunohistochemistry. Mitosis index is evaluated using histological preparation with HE staining. Sexual activity of old male *R. norvegicus* rats was observed at 01.00 a.m. until 04.00 a.m. after treatment, the mean ± standard deviation difference of the control group is 0.00 ± 0.866[a], the water extract / decoct mean 2.22 ± 1.394[b] and ethanol extract 1.89 ± 1.269[b], Where significant value (p α 0.01).In studies of mitotic index old *R. norvegicus* mice testicular after being given a PSP *G. lucidum* extract ethanol = β Glucan, it had the highest with a mean value of 12.11 ±2.759[c] compared to decoction extract with a mean of 8.89 ± 1.364[b] while at control, the lowest mean is 2.56 ± 1.333[a]. There were significant differences in the average value of testosterone between groups with p<0.0001. There was an increase in the mitotic index between groups, at the group of *G. lucidum* PSP ethanol extract, than the decoction extract of PSP *G. lucidum*.

Keywords: LOH, β Glucan *Ganoderma lucidum*, sexual behavior, Testosterone testes, mitotic index.

INTRODUCTION

Ganoderma lucidum has been one of favorite oriental medicine for centuries. Main fruit body is called *Lingzhi* in China and *Reishi* in Japan, used as traditional medicine for hepatitis, hypertension, chronic bronchitis, asthma bronchial, cancer, and some other conditions(Berovič et al., 2003; Bohet al., 2007). One study showed that plasma antioxidant associated with coronary heart disease biomarker profile increased after consuming *G.lucidum* for 10 days. The long term toxicity of *G.lucidum* in a study conducted by Gao and Han (2008) that shown it is safe to consume its capsule within dose rage of 0.47 g/kg to 1.87 g/kg body weight.

The clinical importance of an accelerated reduction in the testosterone level(Feldman et al., 2002; Morley et al., 1997) remains controversial (Shames et al., 2007). Because of the uncertainty regarding the nature of testosterone deficiency in aging men(Morales et al., 2006),recent guidelines have suggested that so-called late-onset hypogonadism be regarded as a clinical and biochemical state with advancing age, characterized by particular symptoms and a low level of serum testosterone(Wang et al., 2009). Because of the lack of evidence regarding the exact criteria for identifying testosterone deficiency in older men who donot have pathological hypogonadism(Liverman and Blazer, 2004).There is significant decrease in testosterone level at the age of 55 and above compared to 12 years before. In accordance with the increasing age, the testosterone production also decreased, which is known as the aging process. Testosterone is an anabolic hormone. The increase of life expectancy also increases the number of elderly in the future. Thus, the elderly problems also increased(Kaufman and Vermeulen, 2005). Some men have experienced LOH syndrome in their thirties, but with relatively small amount of approximately 5%(Wibowo, 2002). If deduced based on the facts and realities that many factors that contribute to LOH can be found in Indonesia including pollution, workplace burden and life style, then it is possible that LOH are more experienced by men in Indonesia compared to western countries(Wibowo, 2002). Some preliminary studies had shown prevalence of LOH in some area. In Jakarta, around 70.94% respondent experienced LOH(Taher, 2005).

MATERIALS ANDMETHODS

Study Area

This research was done at experimental animal cage of Pharmacy Faculty Widyamandala University Surabaya and the tests were done at Medical Faculty Brawijaya University Malang.

Data Analysis

Thirty (30) *Rattus norvegicus*, aged 18 month-old each, divided in to three groups. Group A (control), group B (Hot water extract 50 mg/kg body weight in 2 ml *Ganoderma lucidum* polysaccharide peptide (ß-glucan)), and Group C(ethanol extract 50 mg/kg body weight in 2 ml *Ganoderma lucidum* polysaccharide peptide (ß-glucan)). Each group consists of ten rats. Rats in group A were subdivided into 2 cages; each cage consists of 5 male rats. The rats in group B were subdivided in 3 cages each cage consist of 3, 3 and 4 male rats respectively. The group C was also subdivided in 3 cages as previously described in group B. One female rat (12 month-old) was added in each cage. All cages activities were closely monitored using CCTV for 14 days. Each group was observed for their sexual activities during 01.00 a.m. until 04.00 a.m. because at the time, the activity and mice sexual frequency is the most. Sexual activity is characterized by the behavior of male rats which rides on the back of the female rats.

At the 21st day post treatment, the rats were sacrificed using ether. In each rat, one testicle was dissected and removed for histology preparation and the other for immunohistochemistry evaluation on HE staining and the other testicle is examined using immunohistochemical test for the testosterone.

RESULTS

Sexual behavior before and after given *G.lucidum* polysaccharide peptide (ß-glucan)

Results of analysis of variance in Table 1, appears that there is a significant difference ($p < 0.05$) increase in sexual behavior after treatment among the three groups. Therefore a further test was conducted to see the different groups using LSD (Least Significant Different). The LSD test gives results that differ from the control group and the group dekok Ethanol, while the decoction groups did not differ significantly with the ethanol group.

During treatment

During treatment, the rats in the control group were only given food and water *ad libitum*. Rats in group B were given hot water extract 50 mg/kg body weight in 2 ml *G.lucidum* polysaccharide peptide (ß-glucan) daily using oral tube for 21 days. Rats in group C were given ethanol extract 50 mg/kg body weight in 2 ml *G.lucidum* polysaccharide peptide (ß-glucan) daily using oral tube for 21 days. After the 5th day, there was one dead rat in each group. So, there were nine rats remain in each group. After 14 days of treatment, on the 15th day all the groups are monitored again for their sexual behavior. The A group was divided into 2 cages (Cage 7 and Cage 8); each cage consists of 5 male rats. The B group was divided in 3 cages (Cage 4, Cage 5, and Cage 6); Cage 4 and Cage 5 consist of 3 male rats; Cage 6 consists of 4 male rats. The C group was also divided in 3 cages (Cage 1, Cage 2, and Cage 3); Cage 1 and Cage 2 consist of 3 male rats; Cage 3 consists of 4 male rats. Within each cage (Cage 1-8) added one female rat (12 months old). Each group was observed for their sexual activities during 01.00 a.m. until 04.00 a.m. using CCTV (Figure 1), because at the time, the activity and mice sexual frequency is the most (Table 2).

Table 1. Different test results sexual behavior before and after treatment in each group using a paired t-test

Group	Observation	N	Mean ± Standard Deviation	P Value
control	before treatment	9	0.44 ± 0.527	1.000
	after treatment	9	0.44 ± 0.527	
Extracts dekok *G.Lucidum*	before treatment	9	0.33 ± 0.500	**0.001**
	after treatment	9	2.56 ± 1.236	
Ethanol Extracts *G.Lucidum*	before treatment	9	0.11 ± 0.333	**0.002**
	after treatment	9	2.00 ± 1.225	

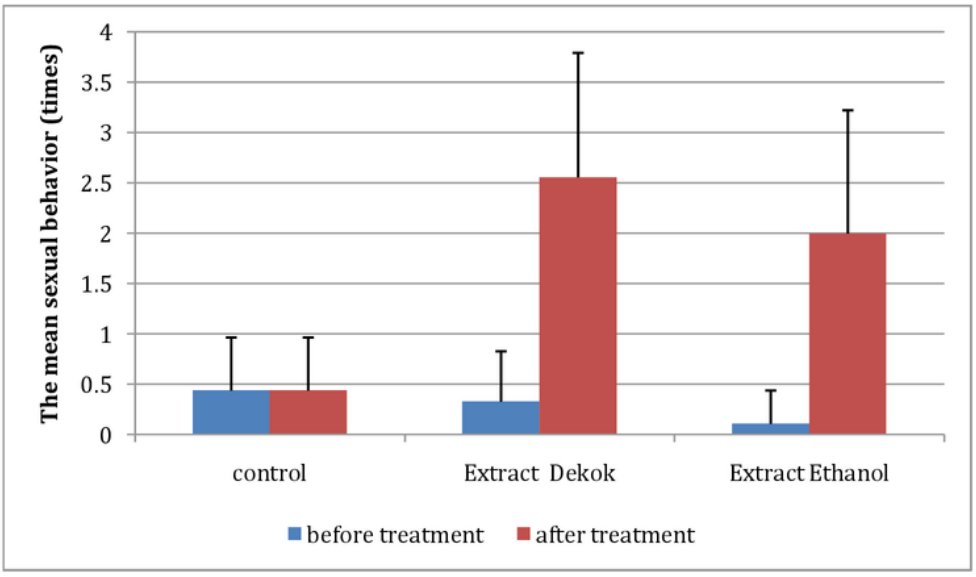

Figure 1. Mean of sexual behavior of *Rattusnovergicus* Before and After Intervention (control group), (extract dekok) and (Extract ethanol)

Table 2. Test results depending on increase of sexual behavior among the three groups after treatment using Variance analysis

Group	n	The mean ± standard deviation difference	p value
control	9	0.00 ± 0.866[a]	
ExtractsdekokG.*Lucidum*	9	2.22 ± 1.394[b]	0.001
Ethanol Extracts *G.Lucidum*	9	1.89 ± 1.269[b]	

Description: Superscript different shows significant differences based on the results of LSD

Leydig Cells profile

Leydig cell profile data to test the normal distribution before in the analysis of the differences between the three groups. The test results showed that the normal distribution of profile data Leydig cells in all three groups had a normal distribution (p> 0.05). Figure 2, Table 3.

Analysis of the differences among the three groups in testosterone performed by analysis of variance

Results of analysis of variance in Table 4. it appears that there is a significant difference (p <0.05) testosterone after treatment among the three groups. Therefore conducted a further test anava to see where the different

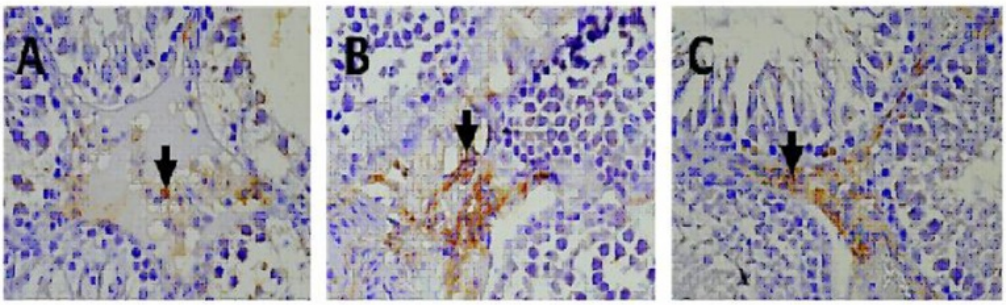

Figure 2. Immunohistochemical staining of testicular Leydig cell hormone testosterone, the control group (A) brown color control (testosterone) less than water extract group (B), and the ethanol extract group (C).

Table 3. Results of normally distributed test laboratory data of each group using the One Sample Kolmogorov Smirnov Test

variables	group	n	p value
testosterone	control	9	0.846
	Extracts dekok*G.Lucidum*	9	0.940
	Ethanol Extracts *G.Lucidum*	9	0.943
Mitotic Index	control	9	0.790
	Extracts dekok*G.Lucidum*	9	0.651
	Ethanol Extracts *G.Lucidum*	9	0.925

Table 4. Results of the analysis of different test Testosterone using Variance

group	n	Mean ± standard deviation	p value
control	9	3.67 ± 1.803^{a}	
Extracts dekok*G.Lucidum*	9	10.89 ± 2.088^{b}	<0.0001
Ethanol Extracts *G.Lucidum*	9	15.44 ± 5.270^{c}	

Description: Superscript different shows significant differences based on the results of LSD test

groups using LSD (Least Significant Different). The LSD test results show that the three groups differ significantly.

Results of analysis of variance in table 5, it appears that there is a significant difference ($p < 0.05$) after treatment of mitotic index among the three groups. Therefore a further test anava was conducted to see where the difference in the groups using LSD (Least Significant Different). The LSD test results show that the three groups differ significantly.

Statistical analysis using One-way ANOVA showed significant difference between control group (A group) and treatment group (B group and C group) ($p=0.00$). There was no difference between the B group and C group. (Figure 3, 4 and 5).

DISCUSSION

Ability, lifestyle and environmental changes that affect reproductive health is an interesting and relevant field of research. Leydig cell is responsible to produce testosterone in mammal's testicle. Testosterone production depends on stimulation of these cells with luteinizing hormone (LH) which is secreted in pulses into peripheral circulation by the pituitary in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus. Testosterone and its aromatase products, estradiol, then provide input back to the hypothalamus and pituitary to suppress the production, on a temporary basis, LH and thus testosterone production. In response to reduced testosterone, GnRH and LH produced again. This negative feedback cycle produces LH pulsatile secretion followed by pulsatile production of testosterone(Bremner et al., 1993; Ellis et al., 1983). During man life cycle, serum testosterone decline usually starts on the fifth decade (Bélanger et al., 1994). The decline usually accompanied with the increase of serum FSH and the increase or no changes in LH level (Zwart et al., 1996). This observation, although they do not abandon deficits related with age from

Table 5. Results of mitotic index difference test using Variance analysis

gruop	n	Mean ± standard deviation	p value
control	9	2.56 ± 1.333[a]	
Extracts dekok*G.Lucidum*	9	8.89 ± 1.364[b]	<0.0001
Ethanol Extracts *G.Lucidum*	9	12.11 ± 2.759[c]	

Description: Superscript different shows significant differences based on the results of LSD test.

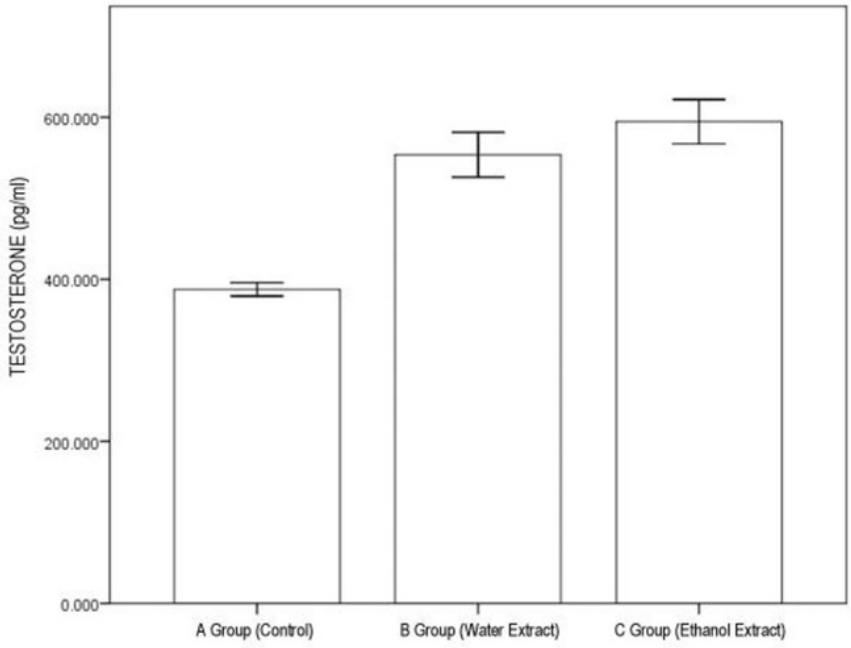

Figure 3. Test results of one-way ANOVA for testosterone three groups after treatment; it turned that testosterone in the treatment group of ethanol extract is higher compared to water extract and control.

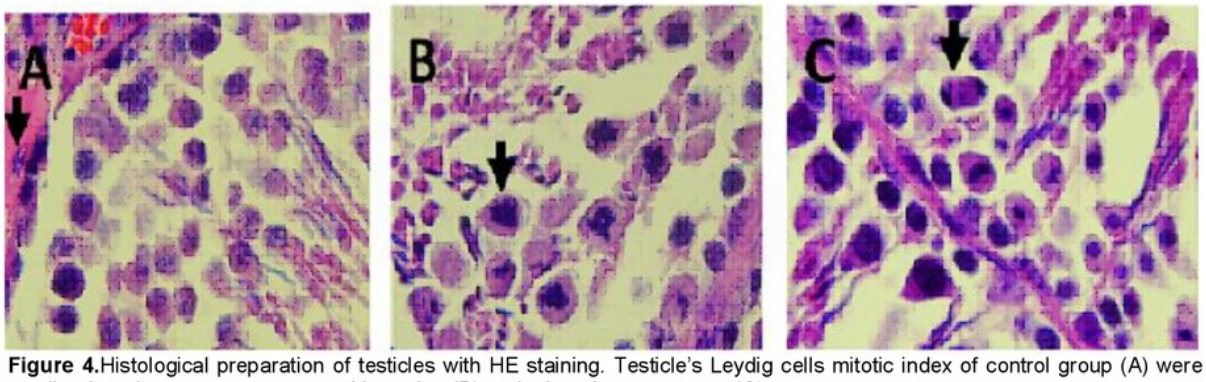

Figure 4. Histological preparation of testicles with HE staining. Testicle's Leydig cells mitotic index of control group (A) were smaller than the water extract group/decoction (B) and ethanol extract group (C)

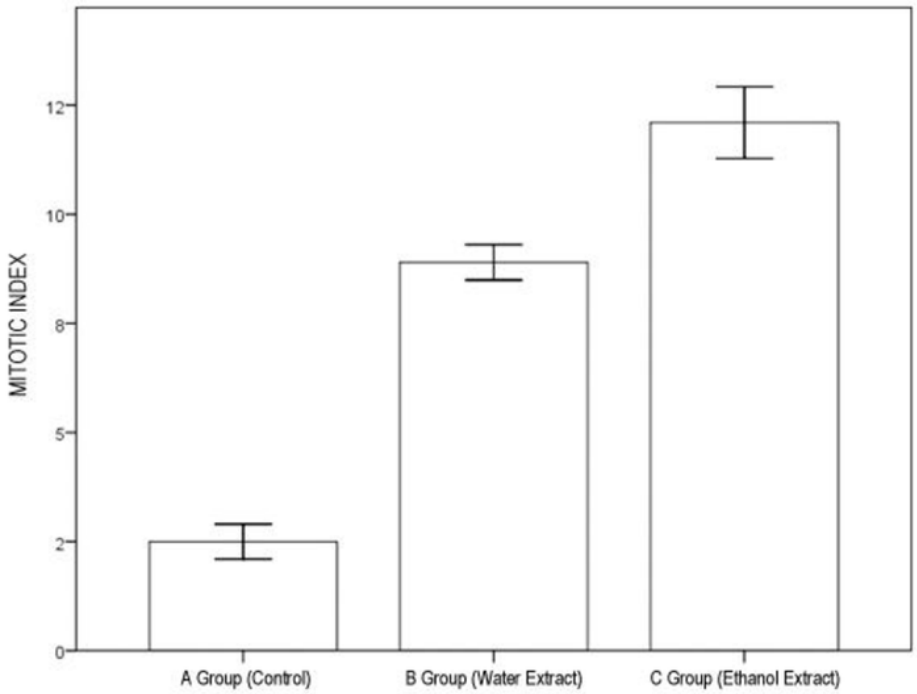

Figure 5. Test results of one-way ANOVA for mitotic index of the three groups after treatment. Mitotic index in the treatment group of ethanol extract is higher compared to water extract and control.

hypothalamus-pituitary axis during human aging, showed a primary testicular deficit. For this purpose, we chose to study aging in Leydig cells of *Rattus norvegicus* rats aged 18 months as a model for humans. In this strain, as in humans, and also other types of mice, the serum levels of testosterone declines with age.

In the preliminary study, one year old male *Rattus norvegicus* was given ethanol extract of *Ganoderma lucidum* polysaccharide peptide (ß-glucan) with different doses: 10 mg/ kg body weight, 20 mg/ kg body weight, and 30 mg/ kg body weight. The study showed significant increase in total testosterone levels in a dose of 30 mg / kg bb, but less than optimal, therefore the dose will be upgraded to 50g mg / kg bb.

Psychological and physiological stress was common, and the study of stress was getting attention. Although the relationship between stress and aging had been known for a long time, theory of aging caused by stress was first proposed in the 1950s(Pare, 1965). This investigation leads to a more general proposal that stress accelerates the aging process on other networks and organs, including the male reproductive system. Stress ability to interfere reproductive function had long been recognized (Marić et al., 1996) and was characterized by decrease in testosterone serum levels(Harman et al., 2001; Vermeulen, 1991). Located in the male testis, Leydig cells were the main source of steroid hormone testosterone. Similar to Leydig cell's aging process, stress inhibits the expression of steroidogenic enzymes and lower the secretion of testosterone (Harman et al., 2001; Matsumoto, 2002; Hardy et al., 2005).

Thirty *R. norvegicus* each was 18 months old, divided in three groups. A group (control group), B group (water extract/ decoct 50 mg/kg body weight in 2 ml *Ganodermalucidum* polysaccharide peptide (ß-glucan)), and C group (ethanol extract 50 mg/kg body weight in 2 ml *Ganoderma lucidum* polysaccharide peptide (ß-glucan) for 21 days. Results analysis of variance in Table 1 appears that there is a significant difference ($p < 0.05$) increase in sexual behavior after treatment among the three groups. Therefore conducted a further test anava to see where the different groups using LSD (Least Significant Different). The LSD test gives results that differ from the control group and the group dekok Ethanol, while the decoction groups did not differ significantly with the ethanol group.

Many studies had shown that apoptosis could occur during aging various populations of cells, including cells of the central nervous system, cardiomyocytes, hepatocytes and lymphocytes; then, aging had been shown to sensitize cells to apoptotic stimuli (Chen et al., 2008). However, in our study to determine testosterone of Leydig cell of *R.norvegicus* testicular rats aged 18-months given ethanol extract of *G.lucidum* polysaccharide peptide (ß – glucan), there was an

increase in testosterone levels tested with imunohistochemical with mean 15.44 ± 5.270[c], compared to extract water / decoction of *G.lucidum* polysaccharide peptide (ß-glucan) with mean testosterone levels is 10.89 ± 2.088[b] and at the control, testosterone levels with mean 3.67±1.803[a]. In conclusion, water extract of *G.lucidum* polysaccharide peptide (ß-glucan) and ethanol extracts could increase level of testosterone of Leydig cells of old *R.norvegicus* testes (18 months) in vivo.

CONCLUSION

G lucidum is a traditional food, especially in the Asian continent, especially the water extract and ethanol extract is evident base which can influence the improvement of male sexual behavior *rattusnorvegicus* by influencing the increase in testicular testosterone. Because this study only lasted 14 days, the results are less than the maximum and therefore the needs to be given *G lucidum* in a longer time, and with a number of samples.

In the study of mitotic index in the testis *rattusnorvegicus* has produced results in the ethanol extract of *G lucidum* extract more significantly compared with water and control.

Disclosure Statement

No competing financial interests exist.

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