SINGLE BULB GARLIC (*Allium sativum*) EXTRACT IMPROVES SPERM QUALITY IN HYPERLIPIDEMIA MALE MICE MODEL

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**ABSTRACT**

The aim of this research was to find out the influence of single bulb garlic on sperm quality improvement in hyperlipidemia model of male mice. Male mice (Balb-C, 12 weeks, bw 21±5 g) were given high fat diet for 45 days until their body weight achieved 45±5 g. The mice were then divided into six groups. The mice in the first group (control group, N) were fed with 7 g high grow pokphan 551 per day. The mice in the second group (control negative group) was fed with 7 g high fat diet (HFD), while the mice in the third group (control positive group) were treated with statin at a dose of 0.91 mg. The mice in groups 4, 5, and 6 were treated with single bulb garlic extract at dose of 125 mg/kg bw, 250 mg/kg bw, and 500 mg/kg bw, respectively. On the 31st day, the mice were dissected and cauda epididymis was taken and chopped in sterile Phosphat Buffer Saline (PBS) and observation conducted on sperm quality consisted of sperm count, sperm motility and sperm normality. The results indicated a significant increase in sperm count, motility and normality in mice treated with single bulb garlic extract at dose of 250 mg/kg bw.

Key words: hyperlipidemia, single bulb garlic, sperm quality

**INTRODUCTION**

Coronary heart disease (CHD) is the highest death cause in the world for all ages. In 2016, World Health Organization recorded an estimation of 17.7 million people died of CHD or 31% of all global dead causes (WHO, 2017). In Indonesia, it is recorded that 12.9% of death are due to CHD making it as the highest cause of death after stroke (Kementerian Kesehatan Republik Indonesia, 2017). CHD main cause is atherosclerosis (Steinberg, 2005; Getz and Reardon, 2007).

Atherosclerosis is one of diseases caused by low density lipoprotein (LDL) oxidative and blockage or plaque thickening in arteries (Tabas, 2004). Plaque thickening inhibits blood circulation to heart (Frostegard, 2013). The causes of atherosclerosis are hypertension, infection (Tabas, 2004; Aziz, 2006; Yunarto and Aini, 2015) and the main cause is high lipid level in blood or hyperlipidemia (Yang and Koo, 2000; Tomkin and Owens, 2012). Hyperlipidemia is a condition where lipid level in blood increases beyond the normal limit and total cholesterol level, triglyceride, and low density lipoprotein (LDL) increase but high density lipoprotein (HDL) decreases (Minich et al., 1997; Almatsier, 2004). Various factors influence hyperlipidemia. The dominant factor is high fat diet (Otunola et al., 2010; Bulqis and Panunggal, 2013). Hyperlipidemia gives negative impact on reproduction system (Chertok et al., 2001; Zhu et al., 2005; Gofur and Lestari, 2016), which is a decrease in sperm quality consisted of sperm count, motility and normality (Ohara et al., 1993; Bashandy, 2006).

Statin is a type of drug often used to cure hyperlipidemia condition (Heart Protection Study Collaborative Group, 2002; Tremblay et al., 2011; Taylor et al., 2013). Statin reduces lipid level by inhibiting 3-hydroxy-3-methylglutaryl Co-enzyme A (HMG-CoA) conversion into cholesterol precursor and mevalonate by inhibiting HMG-CoA enzyme reductase (McKenney, 2003). Continuous statin consumption could bring various negative side effects for body (Golomb and Evans, 2010; Bitzur, 2016; Roy et al., 2017), namely fertility disturbance (Ramirez-Torres et al., 2000) and testosterone production disturbance (Schooling et al., 2013), decreased total sperm count, decreased semen concentration and sperm motility reduction and change the functions of prostatic gland and epididymis (Niederberger, 2005; Pons-Rejrai et al., 2014). Community awareness on the danger of statin encourages the need of natural drug that could lower lipid level in blood, such as single bulb garlic (*Allium sativum*).

Single bulb garlic has been acknowledged as a plant for various diseases (Papu et al., 2014; Iffora et al., 2016). Atherosclerosis is one of diseases caused by low density lipoprotein (LDL) oxidative and blockage or plaque thickening in arteries (Tabas, 2004). Plaque thickening inhibits blood circulation to heart (Frostegard, 2013). The causes of atherosclerosis are hypertension, infection (Tabas, 2004; Aziz, 2006; Yunarto and Aini, 2015) and the main cause is high lipid level in blood or hyperlipidemia (Yang and Koo, 2000; Tomkin and Owens, 2012). Hyperlipidemia is a condition where lipid level in blood increases beyond the normal limit and total cholesterol level, triglyceride, and low density lipoprotein (LDL) increase but high density lipoprotein (HDL) decreases (Minich et al., 1997; Almatsier, 2004). Various factors influence hyperlipidemia. The dominant factor is high fat diet (Otunola et al., 2010; Bulqis and Panunggal, 2013). Hyperlipidemia gives negative impact on reproduction system (Chertok et al., 2001; Zhu et al., 2005; Gofur and Lestari, 2016), which is a decrease in sperm quality consisted of sperm count, motility and normality (Ohara et al., 1993; Bashandy, 2006). Statin is a type of drug often used to cure hyperlipidemia condition (Heart Protection Study Collaborative Group, 2002; Tremblay et al., 2011; Taylor et al., 2013). Statin reduces lipid level by inhibiting 3-hydroxy-3-methylglutaryl Co-enzyme A (HMG-CoA) conversion into cholesterol precursor and mevalonate by inhibiting HMG-CoA enzyme reductase (McKenney, 2003). Continuous statin consumption could bring various negative side effects for body (Golomb and Evans, 2010; Bitzur, 2016; Roy et al., 2017), namely fertility disturbance (Ramirez-Torres et al., 2000) and testosterone production disturbance (Schooling et al., 2013), decreased total sperm count, decreased semen concentration and sperm motility reduction and change the functions of prostatic gland and epididymis (Niederberger, 2005; Pons-Rejrai et al., 2014). Community awareness on the danger of statin encourages the need of natural drug that could lower lipid level in blood, such as single bulb garlic (*Allium sativum*).
al., 2016; Lestari and Rifa'i, 2018) and physiological disturbances (Papu et al., 2014; Lestari et al., 2018). Pharmacological substances containing in garlic consist of allicin (Lawson and Wang, 2005; Rahman, 2007), tannin, alkaloid, various anti-oxidants such as vitamin C, germanium, 33 sulfur compounds, various enzymes, seventeen amino acid, and minerals (Singh and Singh, 2008). The pharmacological substances have various roles in improving hyperlipidemia condition. Anti-oxidant activities in garlic could restrain Cu²⁺-induced oxidation in LDL (Borek, 2001). Allicin could lower blood cholesterol by inhibiting HMG-CoA enzyme reductase activities thus mevalonate cannot be formed that is supposed to be converted into squalen, lanosterol, dihydrolanosterol, D 8-dimethylsterol, 7-dihydrocholesterol and in the end to form cholesterol (Agusti, 1977). Garlic extract could inhibit dependency of cholesterol biosynthesis concentration on various different enzyme stages (14-alpha-demethylase, HMG-CoA reductase) (Handayani, 2006). The inhibition of HMG-CoA enzyme reductase is evidenced by a decrease in blood total cholesterol level (Qureshi et al., 1983). Single bulb garlic benefit has a potential to improve sperm quality in hyperlipidemia model of male mice. The research aimed to find out the influence of single bulb garlic on sperm quality improvement of hyperlipidemia model of male mice.

MATERIALS AND METHODS

The Induction of Hyperlipidemia Mice

Thirty male mice from Balb-C strain, aged 12 weeks and weighed 21±5 g obtained from Malang Murine Farm, Singsosari were acclimated for seven days and fed with 7 g Higrow Pophan 551 and given water. Hyperlipidemia by feeding the male mice with high fat diet (HFD) consisted of 67.2 g duck egg yolk, 0.672 g colic acid, 201.6 g used cooking oil, 134.4 g corn, 66.5 g flour and 201.6 g Higrow Pophan 551. All the ingredients were mashed and formed into circular form weighing 7 g. Feeding high fat diet feeding to mice was carried out for 45 days until the mice weight reached 40 g to 50 g. All research had been approved by the Ethics Commission of Republic Indonesia No. 880-KEP-UB.

The Preparation of Single Bulb Garlic Extract

Single bulb garlic purchased from Tumpang Market, Malang. Single bulb garlic extract was obtained through maceration process by soaking 2 kg garlic into 70% ethanol solvent. The filtrate was evaporated using a rotary vacuum evaporator until it was thick. The extract was kept in a fridge at a temperature of 4°C and did not expose to direct light. Extraction activities carried out at UPT Balai Materia Medica, Batu-Malang.

The Preparation of Simvastatin

One tablet of simvastatin (10 mg) had a mass of 0.2 g; thus, 200 mg tablet contains 10 mg pure simvastatin. Conversion from human dose to mice dose was 0.0026 mg x 10 mg/20 g bw of mice which equal to 0.026 mg for 20 g bw of mice. Dose conversion value was multiplied by mice body weight per 20 g (10 mg) resulted in 0.026 mg. Dose for mice with 35 g bw was converted from 1.75 x 0.026= 0.0455 mg/35 g bw; hence, simvastatin dose per mice was 0.91 mg in 0.5 mL 25% dimethyl sulfoxide (DMSO) per day. Simvastatin application was conducted for 30 days.

Single Bulb Garlic Extract Treatments

The mice were divided into six groups. The first group was the control group (N) treated with 7 g higrow pokphan 551 per day. The second group was the control negative group (Hp), the mice were treated with high fat diet (HFD) at dose of 7 g. The third group was the control positive group (St), the mice were treated with statin at dose of 0.91 mg. The fourth, fifth, and sixth groups were the treatments group and the mice were treated with single bulb garlic extract at dose of 125 mg/kg bw, 250 mg/kg bw, and 500 mg/kg bw, respectively.

Cauda Epididymis Preparation

On the 31st day, mice were sacrificed and dissected and their cauda epididymis was taken. The cauda epididymis was chopped in 1 ml sterile PBS until smooth. Every cauda epididymis was prepared to observe sperm quality consisted of the sperm count, motility, and normality structure.

Sperm Count Observation

Sperm count observation was performed using hemocytometer. The calculation of sperm count was done in five hemocytometer chambers selected under the microscope. The formula for sperm calculation was: sperm count x 10 million sperm cells/mL (WHO, 2010).

Sperm Motility Observation

Sperm motility observation was conducted by categorizing every sperm movement under the microscope. Motility is grouped into five criteria: a) if it moves fast and straight forward, b) if its movement is slow or difficult to move straight forward or does not move straight forward, c) if it does not move forward, d) if it does not move (WHO, 2010).

Sperm Normality Observation

Sperm normality was observed by making smear preparations and then observed the normal and abnormal sperm under a microscope. Normal sperm indicates by the head is bent like a bait, short middle part (middle piece) and long tail (Rugh, 1967).

Data Analysis

Data analysis used one way analysis of variance (ANOVA) and continued with Duncan’s Multiple Range Test (DMRT) to compare the results and observe the differences in every level of single bulb garlic concentration.
RESULTS AND DISCUSSION

Sperm Count

The result indicated that there was a significant difference in sperm count between normal mice, mice that consumed HFD, and single bulb garlic (Figure 1). In reproduction system, lipid in blood, especially cholesterol, is the main substrate to produce steroid hormone (including hormones in reproduction system) (Gwynne and Strauss III, 1982). However, if there is an excess in lipid (hyperlipidemia), it could bring negative impact on sperm as seen in this study. Figure 1 indicated a significant decrease in sperm count in mice fed with HFD. Hyperlipidemia reduces the number of Leydig cells, Sertoli cell function, testosterone roles (Yamamoto et al., 1999) and semen liquid volume (Padron et al., 1989; Monfared, 2013; Schisterman et al., 2014), gonad cells degeneration (Chertok et al., 2001; Zhu et al., 2005), an increase in cholesterol in testis, gonad cells degeneration, hypothalamus-pituitary gland dysfunction, and spermatogenesis disorders (Gofur and Lestari, 2016). As consequence, sperm count produced was small. Drug consumed to lower lipid level was statin.

Statin lower lipid level in blood by inhibiting HMG-CoA conversion into cholesterol precursor and mevalonate by inhibiting HMG-CoA enzyme reductase and it could increase HDL level (McKenney, 2003). Based on the research result, treatment with statin had no impact on the increase in sperm count. However, statin consumption indicated a decrease in sperm count. In addition, statin consumption also raised various side effects on the decrease in sperm quality (Niederberger, 2005; Pons-Rejraji et al., 2014). Hence, natural drug is a necessity, such as single bulb garlic.

The reduction mechanism of lipid level in blood by single bulb garlic was similar to those by statin. Allicin substance containing in the single bulb garlic inhibit HMG-CoA activities (Amagase et al., 2011; Borek, 2001). Treatment using single bulb garlic was proven to be able to significantly increase sperm quality at

Figure 1. Sperm count of male mice after treatments. N= Control group treated with high grow pokpharn 561 of 7 g per day. Hp= Control negative group treated with high fed diet (HFD) at a dose of 7 g. St= Control positive group treated with statin at a dose of 0.91 mg. EBT1= Treated with 125 mg/kg bw single bulb garlic extract, EBT2= Treated with 250 mg/kg bw single bulb garlic extract, EBT3= Treated with 500 mg/kg bw single bulb garlic extract.

Figure 2. Sperm motility after treatments. N= Control group treated with high grow pokpharn 561 of 7 g per day. Hp= Control negative group treated with high fed diet (HFD) at a dose of 7 g. St= Control positive group treated with statin at a dose of 0.91 mg. EBT1= Treated with 125 mg/kg bw single bulb garlic extract, EBT2= Treated with 250 mg/kg bw single bulb garlic extract, EBT3= Treated with 500 mg/kg bw single bulb garlic extract.
doses of 125 mg/kg bw and 250 mg/kg bw. At a dose of 500 mg/kg bw the sperm count did not increase. It indicated that 250 mg/kg bw was the most effective dose to improve sperm quality in hyperlipidemia-model male mice.

Sperm motility was calculated by observing sperm movement. Figure 2 indicated a significant decrease in motility in hyperlipidemia mice. An increase in the number of lipid in blood could cause a decrease in sperm motility (Monfared, 2013). A decrease in motility was also triggered by an increase in reactive oxygen species (ROS) (Henkel, 2011) producing oxidative stress (OS); resulted in, a decrease in sperm motility (Bansal and Bilaspuri, 2011).

Treatment with statin and single bulb garlic significantly increased sperm motility. Both types of drug inhibited HMG-CoA activities (Rai et al., 2009). There was a significant difference in the use of single bulb garlic and statin. The most significant increase occurred in single bulb garlic extract at dose of 250 mg/kg bw. Therefore, the most effective drug to lower lipid level and improve sperm quality was single bulb garlic extract.

Sperm normality was observed in sperm smear apparatus under a microscope. Figure 3 indicated a significant decrease in sperm normality in mice that consumed hyperlipidemia food. Lipid peroxidation could induce morphological changes in spermatozoa (Sanchez et al., 2006). Hyperlipidemia condition influences sperm morphology, especially sperm head (Schisterman et al., 2014). Hyperlipidemia lead to an increase in ROS and OS resulting in morphologically defective sperms (Bansal and Bilaspuri, 2011). Treatment with statin and single bulb garlic could increase sperm normality value. There was only small increase in statin. The most effective sperm normality found in treatment with single bulb garlic extract at dose of 250 mg/kg bb.

CONCLUSION

There was an influence of single bulb garlic extract application on sperm quality improvement in hyperlipidemia male mice model. The most effective sperm quality improvement was found in mice that given 250 mg/kg bw of single bulb garlic extract.

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