Conference Paper

Effect Of *Spirulina Platensis* on The Number of Spermatogenic Cells in The Seminiferous Tubules Of Rat (*Rattus Norvegicus*) with Excessive Physical Exercise

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Abstract

The aim of this study was to investigate the effect of *Spirulina platensis* extract on the number of spermatogenic cells of rats with excessive physical exercise. Excessive physical exercise was achieved through one hour of swimming for 35 days. Twenty male rats were divided into five groups i.e (1) C-, control group, (2) C+, a group was only given swimming, (3) T1, a group was given 300 mg/kg BW of *Spirulina platensis* and swimming, (4) T2, a group was given 600 mg/kg BW of *Spirulina platensis* and swimming, and (5) T3, a group was given 1200 mg/kg BW of *Spirulina platensis* and swimming. *Spirulina platensis* extract was given orally once a day before swimming. ANOVA test followed with Duncan test showed that the number of spermatogenic cells significantly different among treatments ($p<0.05$). Excessive physical exercise was able to influence on the male reproduction system by declined on the number of spermatogenic cells in seminiferous tubules of male rat. The conclusion of this study was dose of 1200 mg/kg BW of *Spirulina platensis* extract could maintain the number of spermatogenic cells of male rat after excessive physical exercise.

Keywords: *Rattus norvegicus*, *Spirulina platensis*, excesive physical exercise, spermatogenic cells.

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1. Introduction

Physical exercise is needed by human and animal to maintain good health, as the result, it can prevent some risk on disease later. Regular physical exercise gives good effect for body which can prevent and treat many chronic disease, such as coronary

heart disease (CHD), hypertension, heart failure, obesity, depression, diabetes mellitus, cancers and osteoporosis (Haskell *et al*., 2007; Oja and Sylvia, 2011), but if the physical exercise is excessive, it can give many disadvantages for body. The excessive physical exercise can inhibit and disturb the physiologic process in body, thereby the homoestasis of body would be interrupted and caused some disease, such as stroke and coroner heart disease (Chevion *et al*., 2003; Hairrudin and Dina, 2009).

Excessive physical exercise induce oxidative stress, included increasing of free radical levels in body. In excessive physical exercise, the free radicals formed primaly are Reactive Oxygen Species (De Lima, 2012). Reactive Oxygen Species (ROS) may attack polyunsatured fatty acids on biomembrane, leading a chain of peroxidation reaction, and alter the membrane structure. This reaction can be defined as lipid peroxidation. Lipid peroxidation produce hydrocarbon gases and malondialdehyde (MDA), which this product is the most frequently sign of increasing ROS (Banerjee *et al*., 2003; Hairrudin *et al*., 2012; De Lima *et al*., 2012).

Testicular membrane is rich in polyunsaturated fatty acid and highly risk to oxidative stress (ROS) which leading lipid peroxidation. This condition may cause disturbance function of testes involved spermatogenesis (Manna *et al*., 2003). Spermatogenesis produce spermatogenic cells included spermatogonia, spermatocyte, spermatid and spermatozoa (Ismudiono *et al*., 2010). Lipid peroxidation can damage polyunsaturated fatty acid of spermatogenic cells, causing dysfunctional spermatogenic cell and decrease quality of seminiferus tubulus (Laksmi, 2010). Lipid peroxidation also reduced motility of spermatozoa, level of plasma testosterone and LH (Manna *et al*., 2003).

Spirulina plantesis is a blue-green alga (cyanobacterium) that has been consumed as food and supplement due to its high contents of proteins, linolenic acid, vitamin and mineral. Many research reported the biological activities of *Spirulina plantesis* as antimicrobial, renoprotector, anti-inflammatory, anticancer, antidiabetic, anti-viral, blood-vessel relaxing effect and hepatoprotective (Mazokopakis *et al*., 2014). In the research of El-Desoky *et al*. (2013) showed protective effect of *Spirulina plantesis* pretreatment could reduce the lipid peroxidation product, mercury accumulation in testis, histopathological changes of testes and spermatozoa abnormalities.

In the present study, an attempt has been made to evaluate the protective potential of *Spirulina plantesis* against excessive physical exercise to testes of male rats by studying the number of spermatogenic cells in seminiferous tubulus.

Table 1: Effect of *Spirulina platensis* on the number of spermatogenic cells in seminiferous tubules of male rat with excessive physical exercise (Mean ± SD).

Treatment	Spermatogonia	Primary Spermatocyte	Spermatid
C (+)	30.27 ± 2.86^{a}	25.15 ± 4.03^{a}	21.05 ± 3.46^{a}
C (-)	62.65 ± 6.47^{c}	51.25 ± 5.07^{c}	76.28 ± 14.88^{c}
T1	42.55 ± 6.19^{b}	39.63 ± 5.82^{b}	45.38 ± 11.88^{b}
T2	46.47 ± 1.41^{b}	42.00 ± 5.18^{b}	47.02 ± 11.86^{b}
T3	56.10 ± 2.34^{c}	50.65 ± 3.98^{c}	75.42 ± 18.88^{c}

Different supercript in the same column indicate significant differences ($P < 0.05$); C – = rat received Na-CMC; C + = rat received Na-CMC+swimming; T1 = rat received 300 mg/kg bw of *Spirulina platensis* + swimming; T2 = rat received 600 mg/kg bw *Spirulina platensis* + swimming; T3 = rat received 1200 mg/kg bw *Spirulina platensis* + swimming.

2. Research Method

The experimental unit used in this study were 20 healthy male rats (*Rattus norvegicus*) at age of 2-3 month, and the weight about 150-200 grams. For excessive physical exercise in this study, rats were swam in a bucket with diameter 35 cm, water depth of 20 cm and water temperature of 32◦C for 60 minutes daily (Bhinekada, 2002).

Twenty male rats were divided into five groups. C- groups were received Na-CMC solution, C+ groups were received swimming, T1 were received 300 mg/kg bw of *Spirulina platensis* extract and swimming, T2 were received 600 mg/kg bw of *Spirulina platensis* extract and swimming and T3 were received 1200 mg/kg bw of *Spirulina platensis* extract and swimming. The treatments were administered using 3 ml of syringe with feeding tube by intragastric gavage. Experimental treatment were done everyday for 35 days. 24 hours from the last treatment, all rats were sacrificed by ether anesthesia and the testes were collected.

Evaluation method by counting four different pieces of testicular section from each treatment. Counting were performed on five different seminiferous tubules for each piece of view starts from the corner of the left, right, top, bottom and middle part of the preparations histology.

3. Result and Discussion

Analysis of variance test followed with Duncan test had been done to see significant differences between treatment after counting the number of spermatogenic cells in seminiferous tubules male rat.

The result showed that spermatogenic cells of C+ groups had significant differences with C- groups, T1, T2 and T3 groups ($p<0.05$). C- groups had significant differences with T1 and T2 group ($p<0.05$). T1 group had no significantly different with T2 group ($p>0.05$). T3 groups had significant differences with T1 and T2 group ($p<0.05$), but had no significant differences with C- group ($p>0.05$).

A group was only given swimming showed the declined of spermatogenic cells in seminiferous tubules male rat (Table 1, Figure 1). It evidenced that excessive physical exercise influenced on male fertility by declined on the number of spermatogenic cells in seminiferous tubules of male rat.

Excessive physical excercise could affect endocrine systems through surpressession of CRH to GnRH leading inhibition in anterior pituitary, which was a potent negative regulator of FSH and LH production (Mastorakos *et al.*, 2005). FSH and LH were gonadotropin hormone stimulating spermatogenesis in male reproductive system (Sonjaya, 2012). In addition, excessive physical exercise also affected in production of testosterone leading low concentrations of testosterone. Testosterone itself had important role in growth and development of male reproductive organs, in association with FSH, acts on seminiferous tubules to initiate and maintain spermatogenesis (Saraswathi *et al.,* 2012).

The declined of spermatogenic cells could be associated with enhacement of concentration of ROS causing lipid peroxidation in testicular membrane which was rich of polyunsaturated fatty acid (Manna *et al.*, 2003). High rate of lipid peroxidation could induce apoptosis, leading cell damage (Ayala *et al.*, 2014). The elevation of MDA (ROS product) also could be associated with the reduction of testicular antioxidant scavenger enzyme, such as SOD, CAT, GPx, GST and GSH leading less protection of enzymatic antioxidant in testicular membrane against free radicals (Manna *et al.*, 2003).

In the present study, result of groups which received *Spirulina platensis* extract and swimming showed enhacement of spermatogenic cells comparing postive control (Table 1, Figure 1). A group received 300 mg/kg bw and 600 mg/kg bw of *Spirulina platensis* extract and swam for 60 minutes had siginificant difference with group which only swam for 60 minutes (Table 1, Figure 1), but it could not maintain the number of spermatogenic cells in seminiferous tubules comparing with normal group (Table 1, Figure 1). It could be indicated that dose of 300 mg/kg bw and 600 mg/kg bw of *Spirulina platensis* extract were not sufficient to protect spermatogenic cells from excessive physical exercise. Meanwhile, a group received 1200 mg/kg bw of *Spirulina platensis* extract and swam for 60 minutes had no significant differences with normal (Table 1, Figure 1). It could be indicated that dose of 1200 mg/kg bw of *Spirulina platensis*

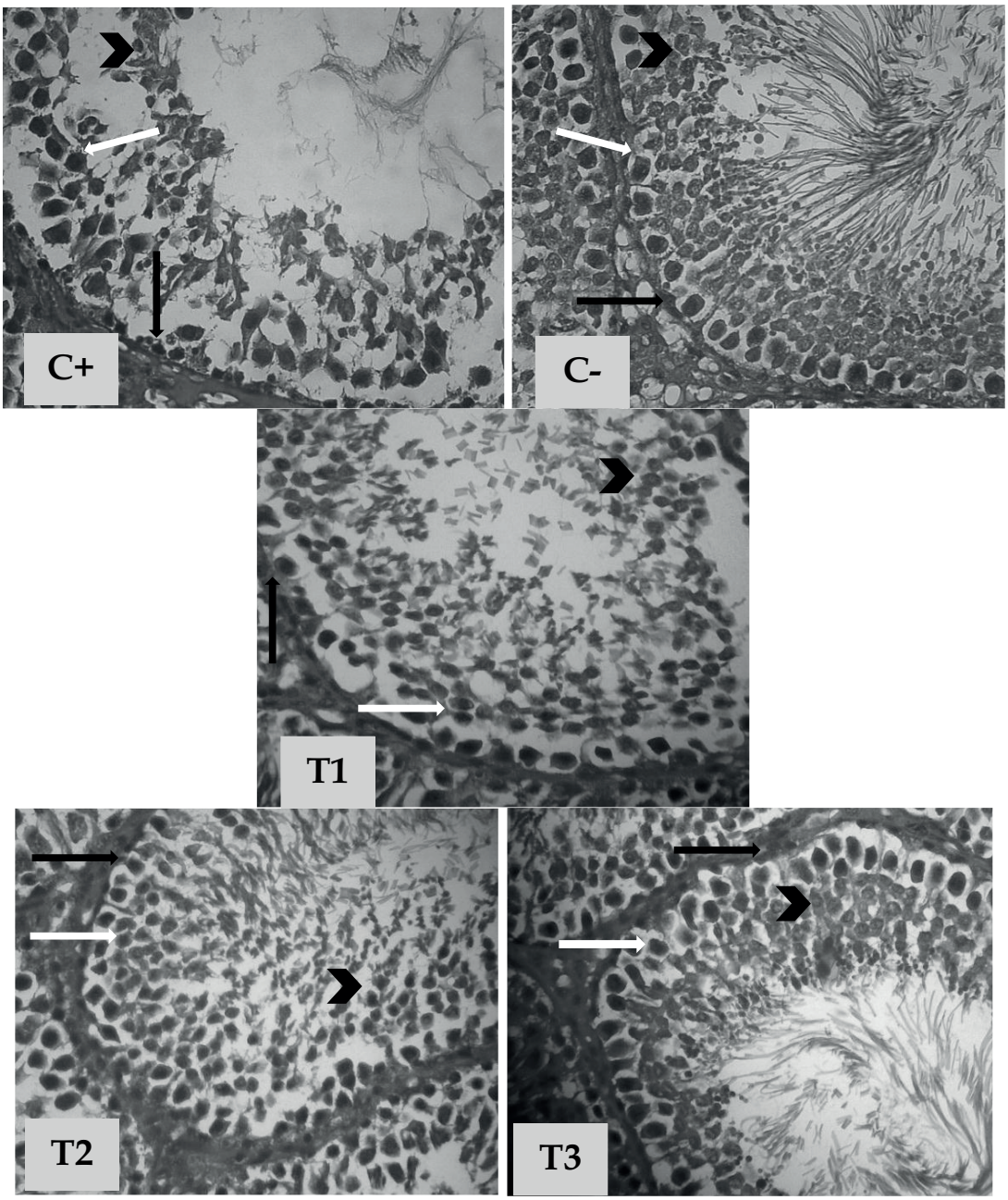

Figure 1: Comparison of histopathological features on seminiferous tubules of rat (*Rattus norvegicus*) in each groups. Black arrows show the spermatogonia, white arrows show primary spermatocyte, and short arrows show early spermatid. C-= rat received Na-CMC (normal seminiferous tubules), C+= rat received Na-CMC+swimming (spermatogenic cells reduced), T1= rat received 300 mg/kg bw of *Spirulina platensis* +swimming (increased of spermatogenic cells), T2 = rat received 600 mg/kg bw of *Spirulina platensis*+swimming (increased of spermatogenic cells), T3= rat received 1200 mg/kg bw of *Spirulina platensis*+swimming (full of spermatogenic cell) (Stain: H.E.; 400x magnification).

extract could maintain on the number of spermatogenic cells in seminiferous tubules male rat.

Potency of *Spirulina platensis* to maintain on the number of spermatogenic cells might be associated to the presence of antioxidant activity. The antioxidant and protective effects of *Spirulina platensis* was owed to their content of antioxidant active constituent such as C-phycocyanin, β-carotene, vitamins, mineral, protein, lipids and carbohydrates (Chu *et al*., 2010).

C-phycoyanin was the most abundant natural antioxidant from *Spirulina platensis*, which could scavenge free radicals by adding one atom hidrogen (Estrada *et al*., 2001). It had been shown that C-phyocyanin could prevent cellular damage occuring as a result of oxidative stress in spermatogenic cells of seminiferous tubules and leydig cells of the stroma (Shaikh *et al*., 2013).

β-carotene from *Spirulina platensis* also had important role to maintain cells from oxidative stress, which could transfer exccessive electron of radical compund into ground state without any chemical change to the β-carotene. It also reacted with peroxyl radicals that were involved in the oxidation of lipids (Al-Attar, 2009). Previous study of Orazizadeh *et al*.(2014) showed that supplementation of β-carotene for 35 days in mice induced by titanium oxide nanoparticles could protect spermatogenesis and cell loss to activity ROS.

Vitamin E and vitamin C in *Spirulina platensis* could decrease lipid peroxidation in testis. Vitamin E was a chain-breaking antioxidant that prevent the propagation of free radicals in membrane and in plasma lipoprotein. When peroxyl radicals were formed, these react 1000 times faster with vitamin E than with polyunsaturated fatty acid. Meanwhile, vitamin C had the ability to protect against lipid peroxidation by one-electron reduction of lipid hydroperoxyl radicals (Traber and Stevens, 2011).

Previous study of El-Desoky *et al*. (2013) showed that *Spirulina platensis* was able to maintain enzymatic antioxidant,such as SOD, CAT and GPx induced mercuric chloride. SOD and CAT scavenged both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. In order to act against H_2O_2, SOD must be conjugated with CAT or GPx. GPx was able to forms an excellent protection against lipid peroxidation of plasma membrane of spermatozoa. It also scavenge H_2O_2, which was responsible for the initiation of lipid peroxidation (Dare *et al*., 2014).

4. Conclusion

Based on this research, it could be concluded that excessive physical exercise could influence of male reproduction by reduction on the number of spermatogenic cells in seminiferous tubules of male rat. *Spirulina platensis* extract could maintain on the

number of spermatogenic cells in seminiferus tubules of male rat (*Rattus norvegicus*) with excessive physical exercise. The best dose in this research was 1200 mg/kg bw of *Spirulina platensis* due to its capability to provide the best protection in spermatogenic cells against excessive physical exercise compared to dose of 300 mg/kg bw of *Spirulina platensis* and 600 mg/kg bw of *Spirulina platensis*

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