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RESEARCH ARTICLE

The Administration of Orange Sweet Potato (Ipomoea Batatas L.) Extract Increases Sperm Consentration, Mortility and Viability in Male Mice Exposed to Cigarette Smoke

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ABSTRAK

Pendahuluan: *Ipomoea batatas L* (IPL) mengandung antioksidan betakaroten, vitamin C, dan vitamin E. Rokok mengandung ROS yang terbukti menurunkan kualitas spermatozoa. **Tujuan:** untuk membuktikan bahwa pemberian IPL dapat memperbaiki jumlah, motilitas, dan viabilitas spermatozoa yang dipapar asap rokok.

Metode: Penelitian eksperimental dengan *post test only control group design,* sebanyak 30 ekor mencit jantan, dibagi menjadi 6 kelompok. Kelompok normal (Nor-G), tidak ada interfensi; Kelompok kontrol negative (Neg-G), hanya dipapar asap rokok; Kelompok kontrol positif (Pos-G), hanya mendapat ekstrak IPL 16 mg/ml; Kelompok IPL-15, IPL-16, dan IPL-17, masing-masing dipapar asap rokok dan mendapat ekstrak IPL 15 mg/ml, 16 mg/ml dan 17 mg/ml.

Hasil: hasil uji *Post Hoc LSD* menunjukkan bahwa jumlah sperma pada IPL-15, IPL-16, dan IPL-17, lebih tinggi bermakna dibanding Neg-G, $p < 0.05$ dan lebih rendah dibanding Nor-G dan Pos-G, $p < 0.05$. Demikian pula dengan persentase viabilitas sperma. Hasil uji *Mann Whitney* menunjukkan bahwa persentase motilitas sperma pada IPL-15, IPL-16, dan IPL-17, lebih tinggi bermakna dibanding Neg-G, $p < 0.05$. Sedangkan dibanding Nor-G dan Pos-G persentase motilitas sperma pada IPL-15, IPL-16, dan IPL-17, tidak berbeda bermakna, $p > 0.05$.

Kesimpulan: pemberian ekstrak ubi jalar jingga dapat meningkatkan jumlah, motilitas, dan viabilitas spermatozoa mencit jantan yang dipapar asap rokok.

Kata kunci : Asap Rokok, motilitas, viabilitas, konsentrasi spermatozoa, Ipomoea batatas, ubi jalar

ABSTRACT

Introduction: *Ipomoea batatas L (LIP) contains antioxidants beta-carotene, vitamin C and vitamin E. Cigarette smoke containing ROS has been proven to decrease sperm quality.* ***Objective:*** *this study aimed to confirm that orange sweet potato extract increases sperm consentration, motility, and viability in mice exposed to cigarette smoke.*

Methods: *In this experimental study with post test only control group design, 30 male mice were divided into 6 groups. Normal group (Nor-G), no intervention, served as the normal control group. Negative control group (Neg-G) was only exposed to cigarette smoke. Positive control group was only treated with IPL 16mg/ml. IPL-15, IPL-16, and IPL-17 group were treated with IPL 15, 16, and 17mg/ml and exposed to cigarette smoke, respectively.*

Results: *Post Hoc LSD analysis showed that means of sperm concentration and viability in IPL-15, IPL-16, and IPL-17 group were significantly higher compared to that of Neg-G ($p < 0.05$) and were lower compared to that of Nor-G and Pos-G ($p < 0.05$). Mann Whitney analysis indicated that the persentage of sperm motility in IPL-15, IPL-16, and IPL-17, were significant higher compared to that of Neg-G, $p < 0.05$. Whilst compared to that of Nor-G and Pos-G the persentage of sperm motility in IPL-15, IPL-16, and IPL-17, there was no significant differences, $p > 0.05$.*

Conclusion: *treatment of IPL capable of increasing sperm concentration, motility, and viability in male mice were exposed to cigarrette smoke.*

Keywords: *cigarette smoke, sperm motility, that extract of orange sweet potato, beta-carotene, Vitamin C, Vitamin E.*

INTRODUCTION

In 2013, Indonesia had the highest prevalence of adult smoker in South East Asia (Coal *et al.,* 2013). While the concentrations of infertility in Indonesia in 2007 reached 10-15% of the 30 million couples of childbearing age. Various litaratures showed that cigarette smoke plays an important role in the incidence of male infertility. This is because a cigarette contains a lot of carbon monoxide, nicotine, and tar which is a free radical. Excessive free radicals can cause cell damage

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Figure 1. Ipomoea batatas L

due to oxidative stress. Sperm's cell membrane contain unsaturated fatty acids easily attacked by free radicals. Therefore excessive concentration of free radicals in the reproductive tract may lower male fertility mediated by a decrease in sperm concentration, motility (Coal *et al.,* 2013).

Orange sweet potatoes (Ipomoea batatas L/ PL; figure 1) which have been commonly consumed contains beta-carotene, vitamin C, vitamin E, lutein, and Zeaxantin having antioxidant activity (Hasim and Joseph, 2008). Studies conducted by Eva *et al.,* (2012) stated that the addition of 0.002 grams of beta-carotene into 1 mL of solvent significantly affects sperm motility and viability in post-thawed Balinese cattle. Furthermore, studies conducted by Adienbo *et al.,* concluded that administration of purple sweet potato powder at a dose of 80 gram per day for 60 days can increase the motility, sperm concentration and viability of Wistar albino rats. Beta-carotene in the IPL has been shown to be much higher than that of in white and purple potatoes (Choong *et al.,* 2006). It can be assumed that orange sweet potato have a more potent antioksidant activity. However, few studies have been conducted on the effects of extract of IPL to increase sperm consentration, motility and viability in mice exposed to cigarette smoke.

The concentration of exessive free radicals can lead to lipid peroxidation resulted in damaged sperm cell membrane, mitochondria {(membranes and DNA (mtDNA)}, and the DNA of the cell nucleus(IDNA). This is because the sperm cell membrane contains unsaturated fatty acids (PUFA). The administration of IPL containing antiok sidan β carotene, vitamin E, vitamin C, lutein, and zeaxantin is expected to prevent membrane damage and DNA (mtDNA & IDNA) spermatozoa were exposed to cigarette smoke. Beta carotene, is the most abundant compound in the carotenoid with conjugated double bonds responsible for its activity as an antioxidant. Studies suggested that β carotene effecetively decreases chloromethylperoxyl and inhibit oxidation by peroxyl radical and break chain reactions that are lipophilic to prevent lipid peroxidation (LPO) (Myers, 2002). Vitamin C (ascorbic acid) is a water-soluble antioxidant which is extremely potent as a radical scavenger which can also affect expression of genes involved in intracellular redox. Vitamin E (a-tocopherol) is an antioxidant that dissolves fat plays animportant role in protecting the integrity of the cellular membrane of phospholipid bilayer and mitochondria by breaking the chain involved in the reaction of lipid peroxidation and increase the production of enzymes antioxidant scavenger such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat). While zeaxanthin are derivatives of carotenoids with high conjugated double bonds, more potent than vitamin E or carnitine (Ros C, *et al.,* 2010).

Based on the description above, it can be assumed, that the administration of IPL can reduce free radicals caused by cigarette smoke exposure leading to incread sperm concentration, motility and viability.

METHODS

This is an experimental study with post test only control group design. Animals used were male Balb/ c mice aged 2-3 months, obtained from the Faculty of Science Biology Laboratory UNNES Semarang. A total of 30 male mice were divided into 6 groups. The normal group (Nor-G) negative control group (Neg-G) was given distilled water; negative control group (Neg-G), mice were only exposed to cigarette smoke; positive control group (Post-G) received 16 mg/ml IPL; Group IPL 15mg/1 (IPL-15), IPL 16 mg/1 (IPL-16), and IPL 17 mg/1 (IPL-17), each mice gain exposure to cigarette smoke and extract IPL 15, 16, and 17 mg/ml. The treatment was given for 35 days and exposure to cigarette smoke by smoking pump. At the end of the study the mice were terminanted with anesthesia. The ductus epididymis and ductus deferen were subjected to sperm consentration, motility and viability.

Manufacture Orange Sweet Potato Extract

Orange sweet potatoes were washed, peeled and cut then dried to obtain the powder as much as 2121 grams. Furthermore, the maceration for 3 days by using 96% ethanol as much as 4000 ml while occasionally stirring. After maceration, then is filtered using a coarse sieve to obtain filtrate. The filtrate obtained was evaporated with a rotary evaporator at a temperature

Table 1. Mean sperm concentration, motility, and viability in each group

Variable	Group						Sig (p)
	Nor-G N = 5 ± SD	Neg-G N = 5 ± SD	Post-G N = 5 ± SD	IPL-15 N = 5 ± SD	IPL-16 N = 5 ± SD	IPL-17 N = 5 ± SD	
BW (gram)	29.0 ± 1.0	29.6 ± 1.1	29.2 ± 0.8	30.2 ± 1.3	30.2 ± 1.1	30.4 ± 1.1	0250 *
Consentration (Jt)	38.4 ± 5.1	18.4 ± 2.1	35.2 ± 7.2	27.6 ± 5.2	28.2 ± 5.7	28.2 ± 4.1	0000 *
Motility (%)	42.7 ± 1.5	6.0 ± 3.6	39.3 ± 4.3	32.0 ± 11	34.7 ± 11.2	34.0 ± 4.3	0000 **
Viability (%)	82.8 ± 5.8	44.6 ± 3.2	79 ± 4.1	65 ± 2.2	69.8 ± 4.3	68 ± 2.9	0000 *

*** Anova; ** Kurkall Wallis**

of 40 °. extract gained as much as 16.89 grams, in the form of a viscous liquid with a concentration of 100%. Subsequently, the extract of IPL was immeressed into three doses of 15.16, and 17 mg/ml administered orally with day before exposure to cigarette smoke.

Cigarette Smoke Exposure

Cigarette smoke in the mentioned using smoking pump two cigarettes a day for 35 days. Smoking pump connected by a wooden box of 35x25x30cm^3, which is occupied by mice.

Statistical Analysis

Since the data for the concentration and viability of sperm with normal and homegenouse distribution, then they were statistically analyzed by ANOVA, followed by a test Post Hoc LSD Test. As for the motility variable, because the data was not normal and not homogeneous, then the statistical analysis used was *Kruskal-Wallis* followed by *Mann Whitney test.* The result of statistical analysis was considered significant if p <0.05. The research was done after approval from Ethics Commission of Unissula Semarang FK. All data were statitically analyzed.

RESULTS

Before the treatment, the body weight (BW) of mice were determined whether BB mice among the comparable group. Results of statistical analysis ANOVA shows that there was no difference between groups B (p> 0.05). Thus giving treatment with a predetermined dose can be continued. After treatment for 35 days, the means sperm concentration, motility and viability of spermatozoa are presented in table 1.

The highest concentration of spermatozoa was found in the Nor-G group, followed by IPL, IPL-16 and IPL-17, IPL-15, and the lowest in the Neg-G group. The highest percentage of sperm motility were found in Nor-G group, followed by the IPL, IPL-16, IPL-17, IPL-15, and the lowest are in clogs pok Neg-G. While the highest percentage of highest viability were those of Nor-G, followed by the IPL, IPL-16, IPL-17, IPL-15, and the lowest are those of Neg-G. The results of the ANOVA statistical analysis (concentration and viability) and Kruskal Wallis (motility) showed no significant differences between groups (p <0.001).

Sperm Concentration

Statistical analysis Post Hoc LSD showed that the concentration of sperm in Neg-G was significantly lower than that of Nor-G, Post-G, IPL-15, IPL-16, and the IPL-17, P <0.05. Concentration of sperm in Nor-G and Post-G showed no significant difference, p> 0.05. lokewise, the concentration of sperm in the 15th IPL, IPL-16, and the IPL-17 showed no significant diffference (p> 0.05). Sperm concentration of in the Post-G was significantly higher than that of IPL-1 5, IPL-16,and the IPL-17, P <0.05 (Figure 2)

Sperm Motility

Statistical analysis of Mann Whitney test showed that the percentage of sperm motility in Neg-G was significantly lower than that of Nor-G, Post-G, IPL-15, IPL-16, and the IPL-17, P <0.05. The percentage of spermmotility on Nor-G and Post-G was significantly different (p> 0.05). like wise, the percentage of sperm in IPL-15, IPL-16, and the IPL-17 shwowed no significant difference(p> 0.05). While percentage of sperm motility in post-G was higher than that of IPL-15, IPL-16, and the IPL-17, but not significant (p> 0.05) (e figure 3).

Sperm Viability

The result of Post Hoc LSD statistic analysis showed that the percentage of sperm viability in Neg-G was significantly lower than Nor-G, Post-G, IPL-15, IPL-16, and IPL-17 (p <0.05). Percentage of sperm viability in Nor-G and Post-G showed no significant difference (p> 0.05). Similarly, the percentage of sperm

viability in IPL-15, IPL-16, and IPL-17 showed no significant difference (p> 0.05). While the percentage of sperm viability in the Post-G was significantly higher than thaat of IPL-15, IPL-16, and the IPL-17, P <0.05 (figure)

DISCUSSION

The results of this study indicates that the administration of smoke on male mice can reduce the concentration, motility and viability of sperm. This is evident from that the concentration, motility, and viability of spermatozoa at Neg-G were significantly lower than of normal and positive controls. The cause of the decreased sperm quality is likely due to the fact that cigarette smoke contain free radicals (carbon monoxide, nicotine and tar). A wide body of evidence suggests that excessive free adicals can degrade the quality of sperm (Coal *et al.,* 2013). Increased levels of free radicals in the tractus reproduction can cause oxidative stress that can damage the membrane, mitochondria, and DNA from the spermatozoa. Spermatozoa membrane damage due to lipid peroxidation can lead to changes in the morphology and decreased sperm motility. On the tail membrane damage causes a decrease in motility of spermatozoa, while the damage the membrane on the head of spermatozoa causes morphological changes and decrease the acrosome reaction. Lipid peroxidation is characterized by increased levels of MDA, which is a component of cell metabolites produced by free radicals, also will trigger the cell membrane damage that can cause cells to lose their cellular functions in total resulting in loss of viability of sperm (Suryohudoyo, 2000).

In addition, high concentrations of free radicals can also cause DNA damage to both mitochondria and cell nuclei. Damage to mtDNA can lead to decreased spermatozoa motility associated with the production of low adenosine triphosphate (ATP), so that the spermatozoa are unable to move (Aryosetyo, 2009). While iDNA damage triggers apoptosis and causes death and decreased spermatozoa viability (Agarwal *et al.,* 2005). When apoptosis occurs in spermatogonia, the sperm concentration increases followed by a decrease in the concentration of sperm due to failure of spermatogenesis (Nasihun, 2010). This supports a study reported by Coal *et al*, which shows that exposure to cigarette smoke on male mice (Mus musculus) decrease the concentration of sperm (Coal *et al*, 2013).

The results of this study also showed that the concentration, motility, and viability of spermatozoa in all groups receiving IPL was better than that of negative group. This illustrates that the provision of IPL with a dose of 15, 16, d an 17 mg/ml can improve the concentration, motility and viability of spermatozoa. Since IPL contain beta-carotene, vitamin C and vitamin E as an antioxidant, it Improve the quantity, motility and viability of spermatozoa due administration deduced IPL comes from these antioxidants. Evidence suggests that sufficient concentration of antioxidants in the body including the reproductive tract can prevent oxidative stress and apoptosis sperm cells caused by free radicals. Beta-carotene, an antioxidant fat-soluble reactive oxygen species can capture and peroxyl radicals,Esvandiary, 2007), thus prevent lipid peroxidation and maintain the stability of cell membranes of spermatozoa (Hidajat, 2005; Myers, 2002). In addition, betakaroten and vitamin C can also bind lipid radicals as a result of lipid peroxidation, thus prevent chain reaction. As a result, the concentration of free radicals decreases and subsequent lipid peroxidation process can be thwarted. Thus, spermatozoa membrane damage that affect the motility, viability and sperm concentration can be prevented (Suryohudoyo, 2000).

In proteins, vitamin C will prevent oxygen reactions with amino acids to form peptides. While the DNA, vitamin C will prevent DNA reaction with oxygen so there is no DNA mutation (Sharma, 2007). Vitamin E is an antioxidant lar ut fat and easily provide h i of hydrogen, of the hydroxyl group in the ring structure to free radicals (Almatsier, 2009). Vitamin E acts as a hydrogen ion donor and can turn into a radical peroxyl radical tocopherol less reactive and more stable (Wijaya, 1996). Vitamin E resides in the cell membrane phospholipids thus maintaining cell membranes from free radicals (Muhilal, 2004). The results of this study are expected to push the orange sweet potato extract as an alternative to prevent a decrease in the concentration, motility and viability of sperm caused by exposure to cigarette smoke.

Research on the effect of extracts of orange sweet potato on the concentration, motility and viability s permatozoa male mice exposed to cigarette smoke is a pre-clinical study. According to the Food and Drug Monitoring Agency (2000) and by the Food and Drug Administration (2001) in the United States, a new drug from natural materials must be subjected to preclinical testing and clinical trials using a randomized controlled trial design it can be applied to humans. According to Minister of Health RI concentration 1992 761 preclinical test consists of test pharmacodynamics and toxicity tests (acute, sub-acute, chronic and specific). Pharmacodynamic test to provide information about the efficacy of an ingredient as well as toxicological test to assess the safety and spectrum of toxic effects of a

material. According Kartasapoetra (1989) factors that affect the nature and content of active ingredients in a plant is determined by genetic and environmental factors. Environmental factors include sunlight, temperature, season, place or area of growth, food substance and maturity level. This study uses orange sweet potatoes from areas of Semarang. Thus, more studies to compare the effectiveness of orange sweet potato extract from various areas on concentrations, motility and viability of spermatozoa are needed.

CONCLUSION

Orange sweet potato extract at a dose of 15, 16, and 17 mg/ml once a day for 35 days increase sperm consentration, motility and viability in male mice exposed to cigarette smoke.

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