THE EFFECT OF N-ACETYL CYSTEINE TO SPERM MOTILITY, VIABILITY, AND CONCENTRATION OF SPRAGUE DAWLEY RATS WITH NICOTINE INHALATION EXPOSURE

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ABSTRACT

Objective: *To prove and analyze the difference of sperm motility, viability, and concentration of Sprague Dawley rats which were exposed to nicotine inhalation, with rats treated with N-acetyl cysteine (NAC) orally and exposed to nicotine inhalation.* ***Material & Methods:*** *Twenty seven rats were allocated into three group. Control group (C) (aquadest inhalation 1 mL/kgBW/day), treatment group 1 (N) (nicotine inhalation 1 mg/kgBW/day), and treatment group 2 (N-NAC) (nicotine inhalation 1 mg/kgBW/day and oral NAC administration 150 mg/kgBW/day), with all treatment were given for 30 days. Orchidectomy was performed on day 31 to collect semen sample for sperm analysis (motility, viability, and concentration).* ***Results:*** *There was a significant decrease in all parameters from N group compared to C group. Significant increase were found in sperm motility and viability parameters in the N-NAC treatment group compared to N group. While the sperm concentration parameters of the N-NAC group had a non-significant increase compared to N group.* ***Conclusion:*** *Exposure to nicotine inhalation decreased sperm motility, viability, and concentration of Sprague Dawley strain rats, and NAC had a protective effect on sperm of rats which was exposed to nicotine inhalation.*

Keywords: *Nicotine, N-acetyl cysteine, sperm analysis, Sprague Dawley rat.*

ABSTRAK

Tujuan: *Untuk membuktikan dan menganalisis perbedaan motilitas, viabilitas, dan konsentrasi sperma pada tikus strain Sprague Dawley dengan paparan nikotin inhalasi dan kelompok tikus dengan paparan nikotin inhalasi dan pemberian N-acetyl cysteine (NAC).* ***Bahan & Cara:*** *27 tikus dibagi menjadi tiga kelompok. Kelompok kontrol (C) (inhalasi aquadest 1 mL/kgBB/hari), kelompok perlakuan 1 (N) (inhalasi nikotin 1 mg/kgBB/hari), dan kelompok perlakuan 2 (N-NAC) (inhalasi nikotin 1 mg/kgBB/hari dan pemberian NAC oral 150 mg/kgBB/hari), dengan perlakuan diberikan selama 30 hari. Orchidectomi dilakukan pada hari ke 31 untuk mengambil sampel semen dan dilakukan analisa sperma. Parameter analisis sperma yang diperiksa yaitu motilitas, viabilitas, dan konsentrasi sperma.* ***Hasil:*** *Didapatkan kelompok N memiliki persentase sperma motil, persentase sperma viabel, dan konsentrasi sperma yang lebih rendah secara signifikan dibanding kelompok C. Didapatkan kelompok N-NAC memiliki persentase sperma motil dan persentase sperma viabel yang lebih tinggi secara signifikan dibanding kelompok N, sedangkan konsentrasi sperma kelompok N-NAC lebih tinggi dibanding kelompok N tapi tidak signifikan secara statistik.* ***Simpulan:*** *Paparan nikotin inhalasi menyebabkan penurunan motilitas, viabilitas, dan konsentrasi sperma tikus strain Sprague Dawley, dan NAC memiliki efek protektif terhadap sperma tikus yang terpapar nikotin inhalasi.*

Kata kunci: *Nikotin, N-acetyl cysteine, analisis sperma, tikus strain Sprague Dawley.*

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INTRODUCTION

Smoking is still being a major problem in worldwide despite the global campaign for the negative impact of smoking to the health. World Health Organization (WHO) reported that the prevalence of smoking in male and female aged ≥ 15 years old is declines from 23.5% in 2007 to 20.7% in 2015, but with increasing of prevalence of some countries in 5 years. The higher prevalence in male with estimated total smokers all over the world was 1.1 billion in 2015.[1]

Infertility is disability of sexually active couple without contraception to achieve spontaneous pregnancy within 1 year.[2,3] Infertility in 25-40% cases is caused by male factors, 40-55% cases is caused by female factors, 10% is due to both male and female, and 10% is idiopathic. Therefore male problems contribute to 50% cases of infertility.[2,3] In 30-40% cases of male infertility, no causes was identified (idiopathic male infertility). The Factors that assumed to cause male idiopathic infertility are endocrine disturbance related to environmental pollution, reactive oxygen species (ROS), or abnormality of genetic and epigenetic.[2,3]

Smoking is predicted as causes of impaired semen parameters and sperm function in male infertile population. Smoking may cause oxidative stress (OS), genetic and epigenetic changes which will result in disorder of sperm function and infertility.[4,5] Negative effect of smoking correlate with the dose of exposure. The problem of smoking involve both active smoker and passive smoker.[5]

Many researches aim to identify correlation of smoking and infertility. Smoking produces toxins that predicted impair fertility which are include benzopyrene, nicotine, cadmium and lead.[5] Electronic cigarette (e-cigarette) produce substances with toxic and carcinogenic property which similar to tobacco smoke in lower dose level.[5] Other study which aim to identify the effect of oral nicotine exposure to rats, reports that there is significant decrease of sperm motility and concentration and the decreased sperm parameter improved with nicotine cessation.[6,7] In some studies with nicotine exposure or smoke, shows that nicotine and cotinine (the metabolite of nicotine) are found in a high level in the plasma and semen, and are predicted to correlate with impairment of sperm function.[5,8]

Some clinical studies of antioxidant administration report the positive effect in sperm deoxyribonucleic acid (DNA). Administration of a combination of vitamin A, vitamin C, and glutathione (GSH), also a combination of vitamin A and vitamin E with N-acetyl-cysteine (NAC) result in significantly decrease of DNA damage markers induced by OS.[4] In vitro study, animal and clinical study of NAC administration report the protective effect to sperm toward OS.[9-11]

OBJECTIVE

This study was aimed to identify and analyze the effect of NAC on sperm concentration, viability, and motility of rats which exposed to nicotine inhalation.

MATERIAL & METHODS

We conducted an experimental animal study (post-test only control group design) to identify and analyze the effect of NAC which given orally to sperm parameters of rats which was exposed to nicotine inhalation.

The animal we used in this study were healthy male Rattus norvegicus Sprague Dawley strain 2-3 months of age, 200-350 grams of body weight, provided by animal laboratory of Veterinary Faculty, Universitas Airlangga Surabaya.

The nicotine preparation we used is e-liquid nicotine solution (NicVape, http://www. nicvape. com, Spartanburg,South Carolina) with nicotine concentration 100 mg/mL, and diluted with aquadest (H_2O) to get nicotine concentration of 1 mg/mL. Nebulizer (Onemed Ion Nebulizer) is used to get aerosol form of nicotine. The nebulizer chamber used is sized 60 x 30 x 30 cm, and the nicotine treatment was given on one group.[7] The NAC (Fluimucil®, Laboratoires Zambon) preparation of 600 mg effervescent tablet diluted with aquadest to get concentration of 75 mg/mL. Oral administration performed by gavage procedure.

The study was performed at Veterinary Faculty, Universitas Airlangga Surabaya, with Ethical Clearance from Ethics Commission of Veterinary Faculty, Universitas Airlangga Surabaya.

The left testis and epididymis was removed, the epididymis was dissected from the testis.The epididymis was incised on petri disc with 1 mL of 0.9% NaCl tocollect the semen for microscopic evaluation. Sperm motility was examined immediately. One drop of semenon a microscope slide, one drops of normal saline was added, then covered with a cover slip. The preparation examined under the microscope using x400 objective.[12]

Viability study aim to count percentage of live spermatozoa was done using eosin/nigrosin

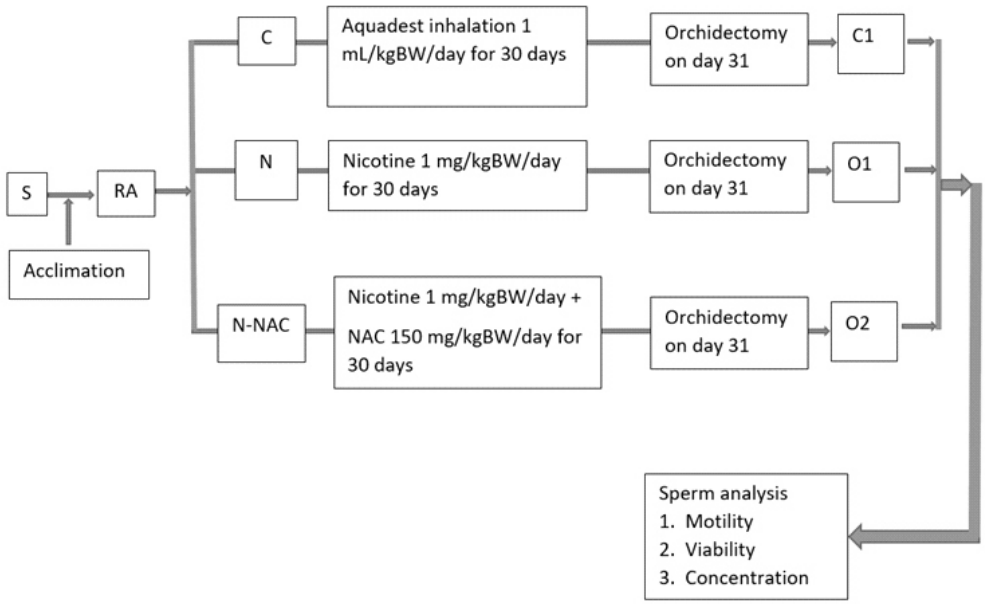

Figure 1. The flowchart showing the study design.

stain preparation. One drop of semen on a microscope slide and one drops of the stain were added. The sperm were counted using x400 objective, where the viable sperm didnot absorb the stain, and the non-viable sperm absorbed the stain.[12]

Sperm concentration count microscopically with improved Neubauer haemocytometer to get sperm count in 1 mL semen. The stained semen is used as the preparation, the sperm were counted using x400 objective.[12]

Experiment was performed with 27 male Sprague-Dawley rats. Acclimation was done for one week. Animals were divided into three equal groups with random allocation (RA). Control group (C) was treated with inhalation of aquadest 1 mL/kgBW/day, nicotine treatment group (N) was treated with inhalation of nicotine 1 mg/kgBW/day, and nicotine plus NAC group (N-NAC) was treated with inhalation of nicotine 1 mg/kgBW/day and gavage of NAC 150 mg/kgBW/day. All group receive treatment for 30 days, and orchidectomy was performed on the 31th day, to collect the semen sample (C1,O1,O2) for sperm analysis (Figure 1).

The Data was analyzed for normality with Shapiro-Wilk test. One way ANOVA is used for analysis if the data has normal distribution, and continued with Least Significant Difference (LSD) Post Hoc test. If the data was not normally distributed, we analyze the data with Kruskall-Wallis test, and continued with Mann-Whitney Post Hoc test. The data presented as mean or median based on normality test, with the difference between the results was considered significant if p value <0.05. The software used for statistical analysis was software statistical product and service solution 25 for Windows (SPSS 25).

RESULT

Research has been conducted to analyze the effect of NAC on sperm concentration, viability, and motility of rats that have exposed to nicotine inhalation. In this study there was no significant difference in body weight between groups before treatment and after treatment as seen in table 1.

In this study the group exposed to nicotine (N) had a significantly lower percentage of motile sperm than the control group (C) as seen in table 2 and figure 2. NACadministration had a significant effect on the percentage of sperm motile sperm cells, where the N-NAC group had significantly higher motile sperm concentrations than N group.

Tabel 1. Body weight comparison of each group.

Body weight	Mean ± SD (g)	p value
Pre-treatment		
Control	286.67 ± 27.39	0.17
Nicotine (N)	263.33 ± 19.37	
Nicotine + NAC (N -NAC)	290.00 ± 34.64	
Post-treatment		
Control (C)	266.67 ± 33.91	0.17
Nicotine (N)	244.44 ± 21.28	
Nicotine + NAC (N -NAC)	268.89 ± 31.80	

Table 2. The median of sperm motility (%) of control,nicotine, NAC, and nicotine with NAC treated group.

Group	n	Median (Min-Max) (%)
Control	9	65.00 (30–75)
Nicotine	9	15.00 (10–30)*
Nicotine + NAC	9	50.00 (20–75)

*p<0.05 differ significantly

Table 3. The mean of sperm viability (%)of control,nicotine, NAC, and nicotine with NAC treated group.

Group	n	Mean ± SD (%)
Control	9	66.67 ± 17.53
Nicotine	9	39.67 ± 12.71*
Nicotine + NAC	9	60.67 ± 15.12

*p<0.05 differ significantly

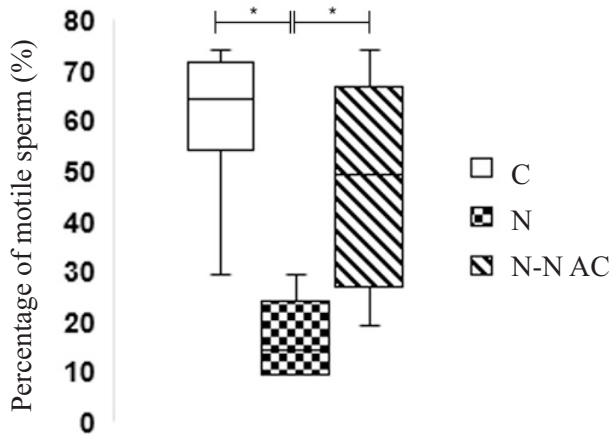

Figure 2. Comparison diagram of the percentage of motile sperm between groups (*p<0.05 differ significantly).

Table 4. The median of sperm concentration (million/mL) of control, nicotine, NAC, and nicotine with NAC treated group.

Group	n	Median (Min-Max) (million/mL)
Control	9	14.06 (12.97–20.63)
Nicotine	9	3.59 (1.56–12.19)*
Nicotine + NAC	9	10.16 (2.19–18.91)*

*p<0.05 differ significantly

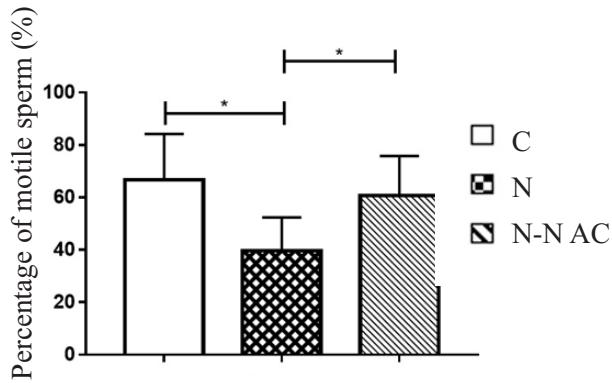

Figure 3. Comparison diagram of the percentage of viable sperm between groups (*p<0.05 differ significantly).

In this study the group N also had a significantly lower percentage of viable sperm than the control group (C) as seen in table 3 and figure 3. NACadministration also had a significant effect on the percentage of sperm motile sperm cells, where the N-NAC group had significantly higher motile sperm concentrations than N group.

In this study it was found that exposure to nicotine significantly reduced the concentration of sperm as seen on table 4. NAC administration did not give a significant effect on sperm concentration. The

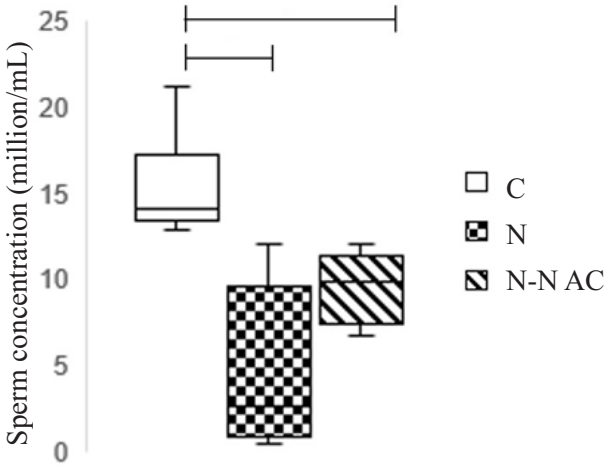

Figure 4. Comparison diagram of the sperm concentration between groups (*p<0.05 differ significantly).

group treated with NAC has a higher sperm concentration compared to the group treated with nicotine alone, but not statistically significant.

DISCUSSION

Nicotine is the component of e-cigarette that cause biological and toxic effect more than other additional components. Nicotine exposure in the form of smoke or vapor from e-cigarette is predicted to have a more efficient pharmacokinetic of nicotine compared to intravenous or oral administration.[13] Nicotine through inhalation will rapidly enter of lung and systemic blood circulation with no intestinal or hepatic metabolism pathway.[13]

The mechanism of nicotine in altering the sperm parameters is predicted by increasing OS in the testicular tissue. OS will cause damages to cell components including DNA, cell membrane, and mitochondria, which results in impaired function and apoptosis of cells. Spermatozoa is very susceptible to the OS because they contain components that are susceptible to oxidation such as polyunsaturated fatty acids, proteins and DNA. Spermatozoa is lack of cytoplasm compared to other cells resulting in a decrease of ability to overcome oxidation reactions and repair the damage that occur.[14] OS can induces the peroxidation of sperm plasma membrane containing lipids, which will reduces membrane integrity, and can also disrupts sperm motility by damaging the axonemal structure. Another mechanism for asthenozoospermia condition is an increase in caspase which is the mediator of apoptosis, thought to cause impaired sperm motility.[4] Nicotine also cause deterioration of gen integrity and expression of testicular cells and was predicted to cause infertility.[15]

The negative effect of nicotine to sperm motility and viability is increased with the higher dose of nicotine given.[16] In some in-vitro study, nicotine shows negative correlation to sperm parameters. Sofikitis et al., reports the level of nicotine metabolite (i.e. cotinine, 3-hydroxy cotinine) in semen plasma have negative correlation with total sperm motility.[17] Condorelli et al., reports the altered of sperm motility in low dose exposure of nicotine to sperm and there was decrease of sperm viability, and increase of apoptosis with fragmented DNA or decreased density of chromatin.[18] Nicotine also induces apoptosis of Leydig cell and inhibits androgen biosynthesis, thus disrupt the male reproduction hormone system.[19]

Other study found that nicotine decreases serum gonadotropin level, causes alteration of spermatogenesis, and decreases antioxidant level of testicular tissue.[8] Nicotine treatment also causes degenerative changes to seminiferous tubule, decrease of spermatogenic cell masses, Sertoli cell vacuolization, and thickening of basal membrane.[20] The negative effect of nicotine is predicted to involve oxidative stress (OS) in testicle tissue and inhibition on reproduction hormone system.[21] Nicotine was predicted to suppress the excretion of gonadotropin from hypophysis which essential for initiation and final stage of spermatogenesis, and decrease steroidogenesis at testis.[22] Nicotine is also decreases germinal cell, Leydig cell, Sertoli cell concentration, and sperm count, alter sperm motility, and increase sperm count with abnormal form. Some study report that nicotine decrease testosterone level through inhibition at some stages of testosterone biosynthesis of rat.[22]

In this study, we found significant decrease of sperm analysis parameters of nicotine treated group. The result is consistent with the previous animal study which report that nicotine treatment decrease the sperm motility, viability, concentration, and increase the sperm count with abnormal morphology. Oyeyipo et al., report that nicotine significantly decrease sperm motility, viability, and serum testosterone, while decrease of FSH occurs at higher dose of nicotine.[6,22]

Antioxidants supplementation is reported to improve the sperm parameters from negative effects related to OS. Treatment by a combination of NAC,

essential fatty acids, vitamin A and vitamin E is reported to reduce sperm DNA damage due to the OS. NAC is also reported to have anti-apoptotic effects, and is known to increase the resistance of sperm toward OS in human semen in vitro.[4] NAC is widely used because it is not toxic, and has the effect of reducing disulfide bonds so that it reduces the viscosity and elasticity of secret or mucus.[23] NAC directly interact with oxidants, as well as GSH, and is a scavenger of hydroxyl radicals. NAC also increases GSH concentration in cells, thereby reducing and preventing cell damage due to oxidants in animal cell cultures.[24] NAC will be hydrolyzed into cysteine, which will binds to glutamate (Glu) and glysine (Gly), then forms glutathione (GSH).[25] GSH is synthesized in the cytosol, also found in the nucleus, and mitochondria, and is a major antioxidant in these parts of the cell.[26,27] GSH in the nucleus is needed for DNA expression and repair processes.[26]

The effect of NAC as an antioxidant was reported in some in-vitro studies. Research by Erkkila et al. demonstrated that NAC administration reduced apoptosis in human testicular germ cells.[10] NAC was a potent scavenger of ROS, increasing GSH concentration in cells, thereby increasing cell defense to OS.[24] NAC was reported significantly reduced ROS in human semen in a in-vitro studies.[10,28]

Animal studies show the protective effects of NAC. In this study, the sperm parameters of the nicotine and NAC treatment groups were higher than the nicotine treatment group, with significant differences in sperm motility and viability parameters. These results are consistent with other studies that show protective effects of NAC related to exposure to ingredients or toxins that cause OS on sperm. Some studies with toxin-exposed animals (paranonyphenol, and glyphosate-based herbicide) and administration of NAC was conducted to prove the protective effect of NAC as an antioxidant. NAC shows protective effect with improvement in sperm parameters, decreased MDA levels in testicular and serum tissue, decreased lipid peroxidation and DNA damage in testicular germ cells.[11,29]

In this study we found an increase in sperm concentration in the group with NAC administration but not statistically significant. This can be due to the effect of suppression of nicotine on gonadotropin secretion which interferes with the reproductive hormone system, and the effects of nicotine which directly interfere with the process of spermatogenesis. Earlier administration NAC before treatment with nicotine, or longer treatment times may show different results.

Clinical studies of NAC administration in male patients show antioxidant effects of NAC. The study by Cifti et al., with NAC 600 mg/ day given orally for 3 months in male patients with idiopathic infertility, result an improvement in semen volume and viscosity, and improvement of sperm motility. The administration of NAC also causes an increase in total antioxidant capacity and decrease in the OS index in the serum.[23] Clinical study by Safarinejad et al., with selenium and/or NAC in infertile men with oligospermia, asthenospermia, or teratospermia results in significant improvement in sperm parameters especially sperm concentration, motility, and morphology.[9] The study also report there was an increase in serum testosterone levels, a positive correlation of selenium and NAC concentrations in serum with sperm parameters, and additive effects from combination of selenium and NAC.[9]

This study only perform quantitative measurement of sperm motility. This study did not conduct the examinations to measure nicotine levels and NAC levels after treatment. This study also did not measure the oxidant and antioxidant parameters in the subject of the study to better explain the mechanism of the negative or protective effects of the treatment given. We cannot conclude of any changes in sperm DNA profile related to this experiment. Another limitation is that there is no histopathological examination testicular cells and level of reproductive hormones associated with the process of spermatogenesis.

CONCLUSION

From previous research and the results of this study showed that exposure to nicotine inhalation decreased sperm motility, viability, and concentration of Sprague Dawley strain rats, and NAC had a protective effect on sperm of rats which is exposed to nicotine inhalation. Further research is needed to gain more evidence about NAC advantages to fertility.

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