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The analysis of giving kebar grass extract (*Biophytum petersianum*) toward malondialdehyde levels and spermatozoa morphology balb/c male mice that exposed By cigarrette smoke

Dwi Ulfa Ningsih[1], Melyana Nurul Widyawati[2], Budiyono[3]
[1] Midwifery Student, Postgraduate Program, Masters Applied of Health, Indonesia
[2] Politeknik Kesehatan Kemenkes Semarang, Semarang, Indonesia
[3] Universitas Diponegoro Semarang, Semarang, Indonesia

Abstract
Background: Exposure to cigarette smoke increases reactive oxygen species, decreases natural antioxidants resulting in damage to cells, tissues and inhibits the process of spermatogenesis. Kebar grass extract contains antioxidants, protecting cells and tissues from damage caused by exposure to free radicals.
Objective: To determine effect of giving different doses of kebar grass extract to the Malondialdehyde levels (MDA) and spermatozoa morphology Balb/C male mice that exposed by cigarette smoke.
Method: The research using a post test only control group design. Total sample of 18 Balb/C male mice divided into 3 groups. All groups were exposed to non-filter cigarette smoke with tar levels of 39 mg and nicotine 2.3 mg. The control group (K) was only given exposure to cigarette smoke. Experiment Group$_1$ (E_1) was exposed to cigarette smoke and kebar grass extract at a dose of 0.75 gr / 25 mL aquades. Experiment Group$_2$ (E_2) was exposed to cigarette smoke and kebar grass extract at a dose of 1.25 gr / 25 mL aquades. The treatment was given for 28 days, on the 29th day all mice were terminated and Malondialdehyde levels (MDA) and spermatozoa morphology were examined.
Results: The results test One Way Anova and Post hoc Multiple Comparisons showed p value = 0.001, so it can be concluded that kebar grass extract influences Malondialdehyde levels (MDA) and spermatozoa morphology of Balb/C male mice that exposed to cigarette smoke with an average MDA level of K group = 7.86 µL / ml; E_1= 5.58 µL / ml; E_2= 3.26 µL / ml and morphology group K = 80.83%; E_1= 88.5%; E_2= 93.66%.
Conclusion: The use a dose of 0.75 gr and 1.25 gr / 25 mL of distilled water for MDA levels has the same effect, but a dose of 1.25 gr / 25 mL of distilled water for morphology has the best results.

Keywords: kebar grass extract (*Biophytum petersianum*), Malondialdehyde levels (MDA), Spermatozoa morphology, Balb/C male mice, Cigarette smoke

1. Introduction

Infertility is a problem that occurs throughout the world and the majority occurs in developing countries [1-3]. One in seven couples experience both male and female infertility, around 20-30% of cases of infertility are caused by male factors in several countries including in Asia. The incidence of infertility as much as 15-25% of cases occur in couples in Indonesia [4, 5]. The cause of infertility in men is characterized by reproductive disorders, poor semen quality and the results of spermatozoa analysis which are critical determinants of conception success [6]. In couples undergoing infertility treatment, 50% are found with abnormal sperm quality and more than 90% of cases of male infertility are due to a low amount of semen [7, 8].

Poor semen quality is closely related to unhealthy lifestyle habits, the environment, work and free radical exposure. Free radicals are found in sperm physiologically, the presence of free radicals in the body accompanied by endogenous defense mechanisms that will produce substances that have an effect as antioxidants. When an imbalance occurs between the levels of ROS (Reactive Oxygen Species) that increase so that it cannot be maintained by natural antioxidant sperm, oxidative stress will occur which will cause cellular damage to sperm. It is proven that 30-80% of spermatozoa quality and poor semen count are caused by the effects of oxidative stress. Oxidative stress has been recognized as one of the most important causes of infertility in men [9, 10].

Decreased semen quality caused by increased ROS production, exacerbated by smoking in men. Smoking causes a combustion reaction that will form nitrogen compounds, carbon monoxide, carbon dioxide and peroxide compounds that trigger free radicals. Nicotine in cigarettes has a vasoconstrictor effect which can cause morphological abnormalities and tar in cigarettes is carcinogenic which can cause impotence. Smoking can reduce the levels of the hormone testosterone which inhibits the process of spermatogenesis and suppress antioxidants in semen that will cause damage to cellular DNA, sperm membrane and disrupt the reproductive system resulting in decreased quality of spermatozoa [11-13].

Smokers have a prevalence of 1.3 billion people worldwide and 80% occur in developing countries. The number of smokers in Indonesia is 34.8%, including men as much as 67.0% and women as much as 2.7%. Indonesia ranks third after China and India. Exposure to cigarette smoke can result in a decrease in the quality of spermatozoa and reduce the partner's 40% chance of getting pregnant naturally. Smoking can also cause low semen volume, decreased sperm count, impaired sperm motility, viability and DNA

compared to non-smokers. ROS levels in semen correlate with the amount and duration of smoking [14-16].

Male fertility parameters were assessed from semen analysis that reflected the volume of semen fluid, the number of spermatozoa, concentration, vitality, motility and morphology. Abnormal sperm quality cannot reach the egg so it can cause the possibility of a decrease in the success of conception [17-19]. Oxidation reactions can be prevented and slowed by intake of foods that contain antioxidants, because antioxidants are compounds that protect cells and tissues from damage due to exposure to free radicals. These antioxidants include vitamin E and vitamin A which are non-enzymatic antioxidants [11, 20].

Conventional treatment is done by infertile couples in an effort to get offspring using assisted reproductive technology therapy or called ART (Assisted Reproductive Treatment), but this therapy has the effect of pain physically and emotionally in each cycle of the treatment carried out so that it affects infertile couples to continue therapy. Infertility therapies include intrauterine insemination, *In vitro* Fertilization (IVF), Embryo Transfer (ET), and Intracytoplasmic Sperm Injection (ICSI). This method is still popular, but relatively high cost, the success rate is not optimal even has a risk of birth defects [9, 18, 19].

Herbal remedies have been used for thousands of years and the trend is increasingly widespread in several countries, even the WHO (World Health Organization) states 80% of the world population, especially in developing countries use herbal medicines for health including infertility and dysfunction treatments sexual. Indonesia has several plants that are used by the community to increase fertility in men, including Kebar grass, red ginger, katuk leaves and black cumin [21-24].

Kebar Grass Extract (Biophytum Petersianum) is also called banondite which means that many children are one of the herbs that grow wild in Central Java, West Java and Kebar Monokwari District, West Papua. Kebar grass from Papua has a higher antioxidant content than Kebar grass from West Java and Central Java. Based on local culture in Papua, people use Kebar grass plants to treat thrush, laxatives in children, antidote to poisons from snake bites and various other health problems, but are more widely used by residents as fertility therapy to improve and maintain reproductive performance because they contain active compounds steroids, saponins, flavonoids, calcium, phosphorus, vitamins A, E and amino acids needed for reproduction [25-27]. Based on the results of research analysis on spermatogenesis of malemice Balb / c given infusion of Kebar grass with multilevel doses of 1%, 3%, 5% and 10% can increase activity in the process of spermatogenesis, but can cause a decrease in spermatogenesis activity at too high concentrations. Calcium, phosphorus and magnesium play a role in the formation of nucleoproteins and are responsible for the formation of cells that affect the mobility of sperm which is very active so that fertilization occurs.[27-30] Other chemical compounds contained in Kebar grass are vitamin A, vitamin E and minerals. Based on research results that vitamin A can restore the morphological normality of spermatozoa in rats exposed to borax. Sperm DNA damaged by oxidation has been shown to be able to be protected with vitamin E, because vitamin E has antioxidant properties as free radicals [13, 28]. Based on the culture and beliefs carried out by people in West Papua that Kebar grass is used as a fertilizer treatment that is consumed especially in women of reproductive age, it is necessary to further prove the effect of giving Kebar grass to the levels of Malondialdehyde and the morphology of spermatozoa of malemice Balb / c exposed to cigarette smoke.

2. Methods

This type of research uses an experimental research design (True Experiment) with a post test only control group design. Researchers arranged three groups consisting of two experimental groups and one control group. In the experimental group was given exposure to cigarette smoke (tar concentration 39 mg and nicotine 2.3 mg / day) and given a kebar grass extract at a dose (0.75 gr / 25 mL aquades and 1.25 gr / 25 mL aquades), while the group controls were given exposure to cigarette smoke with a concentration of 39 mg tar and nicotine 2.3 mg / day and were not given kebar grass extract. The treatment of exposure to cigarette smoke and administration of kebar grass extract was carried out for 28 days. On the 29th day measurements were taken in the experimental group and the control group then a comparison was made in the experimental group with control group. Measurement of levels Malondialdehyde using spectrophotometer instruments and morphological observations using the Nikon ECLIPSE E100 binocular microscope. The population in this research was Balb/C male mice (Mus musculus) obtained from the Biology Laboratory of the Animal Maintenance Unit State of Semarang University as a place for the maintenance of test animals. Determination of the minimum sample size using technique probability sampling with method simple random sampling and is based on the inclusion and exclusion criteria of 18 Balb/C male mice divided into three groups with 12 Balb/C male mice in the experimental group and 6 Balb/C male mice respectively in the control group. In this reseach, researchers conducted data collection by identifying and filling out observation sheets. The data collected was analyzed through the IBM SPSS program version 24.0, and continued with a different test, namely the parametric test (One Way Anova and Post hoc Multiple Comparisons). The processed data is used as a basis for discussing statement matters, which are then presented in tabular form so that conclusions can be drawn.

3. Results

Table 1: Distribution frequency of MDA (Malondialdehyde) *Balb/c* male mice in the control group and treatment group

No.	Group	Sample	MDA Level (µL / ml)	Average MDA level (µL / ml)	ρ value *
1	K	K.1	7.31	7.86	0.748
		K.2	7.56		
		K.3	7.87		
		K.4	8.63		
		K.5	8.44		
		K.6	7.37		

2	E1	E1.1	5.11	5.58	
		E1. 2	5.55		
		E1.3	6.81		
		E1.4	4.86		
		E1.5	5.04		
		E1.6	6.11		
3	E2	E2.1	3.36	3.26	
		E2.2	3.36		
		E2.3	4.23		
		E2.4	3.72		
		E2.5	2.65		
		E2.6	2.28		

*Homogenity of variances: significant >0,05 Description: K (Control group), E_1 (Experiment group dose 0,75 gr/25 mL aquades), E_2 (Experiment group dose 1,25 gr/25 mL aquades).

Based on the table above, shows the highest MDA levels in the control group (K) with no Kebar grass extract and an average of 7.86 µL / ml, while the lowest MDA levels are in the group (E_2) with the administration of Kebar grass extract with a dose of 1.25 gr / 25 mL of distilled water and an average of 3.26 µL / ml. The test results found that MDA levels in the control group and treatment group were homogeneous with a ρ value of 0.748.

Table 2: Distribution frequency spermatozoa morphological *Balb/c* male mice in the control group and treatment group

No.	Group	Sample	Percentage of spermatozoa morphology (%)	Mean percentage of spermatozoa morphology (%)	ρ value *
1	K	K.1	78	80.83	0.53
		K.2	72		
		K.3	77		
		K.4	84		
		K.5	86		
		K.6	88		
2	E1	E1.1	88	88.5	
		E1.2	85		
		E1.3	88		
		E1.4	89		
		E1.5	93		
		E1.6	88		
3	E2	E2.1	96	93.66	
		E2.2	96		
		E2.3	92		
		E2.4	91		
		E2.5	94		
		E2.6	93		

*Homogenity of variances : significant >0,05 Description: K (Control group), E_1 (Experiment group dose 0,75 gr/25 mL aquades) E_2 (Experiment group dose 1,25 gr/25 mL aquades.)

Based on the table above, shows the highest spermatozoa morphology in group E_2 by giving Kebar grass extract a dose of 1.25 gr / 25 mL aquades and an average of 93.66%, while the lowest spermatozoa morphology of percentage in the control group (K) with no grass stiff and an average of 80.83%. The test results showed that the spermatozoa morphology in the control group and treatment group was homogeneous with a ρ value of 0.53.

Table 3: Effects kebar grass extract (*Biophytum petersianum*) on MDA levels *Balb/c* male mice between in the control group and treatment group

No.	Group	ρ value*	ρ value* (PH Test)
1	K (Control group)		K against E1
2	E1 (Treatment group dose 0.75 gr)		K against E2
3	E2 (Treatment group dose 1.25 gr)	0.001	E1 to E2 0.001

*ρ value and Post Hoc Test: significant <0.05

Based on the table above, shows that the administration of kebar grass extract (*Biophytum petersianum*) effect on MDA levels with a ρ value of 0.001 (ρ <0.05) through the test One Way Anova. In addition there were significant differences in MDA levels between control groups (K) not given kebar grass extract with the treatment group (E_1) kebar grass extract at a dose of 0.75 gr / 25 mL aquades. The control group (K) was not given kebar grass extract with treatment group (E_2) kebar grass extract dose of 1.25 gr / 25 mL aquades. Treatment (E_1) on treatment (E_2). This was proven through Post Hoc Bonferroni Test with a ρ value of 0.001 (ρ <0.05).

Table 4: Effects kebar grass extract (*Biophytum petersianum*) on the spermatozoa morphology *Balb/c* male mice between in the control group and treatment group

No	Group	ρ value*	ρ value* (PH Test)
1	K (Control group)	0.001	K against E2 0.001
2	E1 (Treatment group dose 0.75 gr)		
3	E2 (Treatment group dose 1.25 gr)		

*ρ value and Post Hoc Test: significant <0.05

Based on the table above, shows that the administration of kebar grass extract (*Biophytum petersianum*) has an effect on the spermatozoa morphology with a ρ value of 0.001 (ρ <0.05) through the test One Way Anova. In addition there were significant differences in spermatozoa morphology between in the control group (K) not given kebar grass extract with treatment group (E_2) kebar grass extract at a dose of 1.25 gr / 25 mL aquades. This was proven through the Post Hoc Bonferroni Test with a ρ value of 0.001 (ρ <0.05).

4. Discussion

4.1 Measurements MDA level and observations spermatozoa morphological of *Balb/c* male mice between in the control group and treatment group

The results of the study showed that MDA levels between in the control and treatment groups were homogeneous (ρ value = 0.748) and data on MDA levels between the control groups and treatment group were normally distributed (ρ value> 0.05) with a ρ value of 0.308, where the average percentage of MDA levels in the control and treatment groups differ. The treatment group that was given kebar grass extract (*Biophytum petersianum*) showed a decrease in the percentage of MDA levels compared to the control group that was not given kebar grass extract.

The results showed that the spermatozoa morphology between groups was homogeneous (ρ value = 0.53) and the spermatozoa morphological data between in the control group and treatment groups were normally distributed (ρ value> 0.05) with a ρ value 0.126, where the average spermatozoa morphology in the control group and treatment groups vary. The treatment group given kebar grass extract (*Biophytum petersianum*) showed an increase in the spermatozoa morphological percentage that higher than the control group.

Spermatozoa morphology is a very important predictor in the functional spermatozoa to achieve fertilization. Fertilization will occur if the spermatozoa have a complete head, body and tail so that the spermatozoa are able to move quickly and survive to fertilize the egg, but if the spermatozoa is abnormal, it will affect the mobility that causes the low quality and functional ability of the sperm to fertilize the egg.

This condition shows that the presence of phosphorus and magnesium which plays a role in the formation of nucleoproteins responsible for cell formation that affects the mobility of highly active spermatozoa resulting in fertilization and the presence of calcium contained in the Kebar grass extractactive (Biophytum petersianum) is known to play a role in helping the movement of spermatozoa into theOvum [29].

4.2 Effect kebar grass extract (*biophytum petersianum*) toward spermatozoa morphology and MDA levels on *Balb/c* male mice between in the control group and treatment group

The results showed the administration of Kebar grass extract (*Biophytum petersianum*) affected in the MDA levels and spermatozoa morphology *Balb/c* male mice with a ρ value of 0.001. The potential of kebar grass extract (*Biophytum petersianum*) on MDA levels shows that there are significant differences in MDA levels between in the control group and treatment group, groups (E_1) against (E_2) with ρ value of 0.001 through Post Hoc Bonferroni test with ρ values 0.001 (ρ <0.05). This shows that the kebar grass extract (*Biophytum petersianum*) dose 0.75 gr / 25 mL aquades and 1.25 gr / 25 mL aquades have the same effect on MDA levels in *Balb/c* male mice.

The potential of kebar grass extract (*Biophytum petersianum*) on the spermatozoa morphology shows that there are significant in differences spermatozoa morphological between in the control group of (E_2) with a ρ value of 0.001 (ρ <0.05). This shows that kebar grass extract (*Biophytum petersianum*) dose of 1.25 gr / 25 mL aquades has a better effect on the spermatozoa morphology of *Balb/c* male mice.

Significant differences in MDA levels and spermatozoa morphology in the control and treatment groups were known because of the presence of free radicals due to exposure to cigarette smoke. Free radicals can cause damage to cells and normal body tissues so antioxidants are needed to prevent or slow down oxidation reaction. The presence of non-enzymatic antioxidants (vitamins A and E), calcium, phosphorus and amino acids in the Kebar grass extract (Biophytum petersianum) is thought to play a role in counteracting free radicals due to exposure to cigarette smoke.

Exposure to cigarette smoke is responsible for increasing DNA damage, a study shows that a decrease in the quality of spermatozoa among male smokers correlates with an increased level of DNA fragmentation.[12] Nicotine in cigarettes has a vasoconstrictor effect resulting in morphological abnormalities of spermatozoa. Smoking can also reduce the levels of the hormone testosterone which inhibits the process of spermatogenesis and suppress antioxidants in semen that will cause damage to cellular DNA, sperm membrane and disrupt the reproductive system resulting in decreased quality of spermatozoa. This is offset by the presence of flavonoid compounds which can also increase the amount of the hormone testosterone through the inhibiting mechanism of the hormone progesterone. The performance of flavonoids in the body is also able to bind to alpha estrogen receptors (REα) in the testis and epididymis so that it can replace estrogenic function and work together with testosterone for spermatozoa maturation [29].

A large meta-analysis involving men from 26 countries / regions concluded that smoking caused a decrease in sperm quality in infertile and infertile men. Sperm concentrations in male smokers were reported to be typically 13-17% lower than nonsmokers. Decreased semen quality was found more in heavy smokers (> 20 cigarettes / day) and moderate (10-20 cigarettes / day) compared to light smokers (1-10 cigarettes / day). Smoking has a damaging effect on motility, viability, spermatozoa DNA and directly correlates with the amount and duration of smoking [31].

Thus, the general effect of smoking on male fertility can result from a combined role of increased oxidative stress, DNA damage, and cell apoptosis which can not only reduce the quality of spermatozoa, but also interfere with spermatogenesis, sperm maturation, and spermatozoa function.

5. Conclusion

The results of research conducted on 18 *Balb/c* male mice by administering kebar grass extracts (*Biophytum petersianum*) for 28 days obtained the results:

1. Kebar grass extract (*Biophytum petersianum*) affects in the MDA levels on *Balb/c* male mice spermatozoa exposed to cigarette smoke with 28 days ρ value 0.001.
2. Kebar grass extract (*Biophytum petersianum*) influences in the spermatozoa morphology on *Balb/c* male mice exposed to cigarette smoke with a ρ value of 0.001.
3. Kebar grass extract (*Biophytum petersianum*) dose 0.75 gr / 25 mL aquades and 1.25 gr / 25 mL aquades are equally effective against MDA levels and kebar grass extract (*Biophytum petersianum*) doses 1.25 / 25 mL aquades are more effective against spermatozoa morphology on Balb/c male mice exposed to cigarette smoke.

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