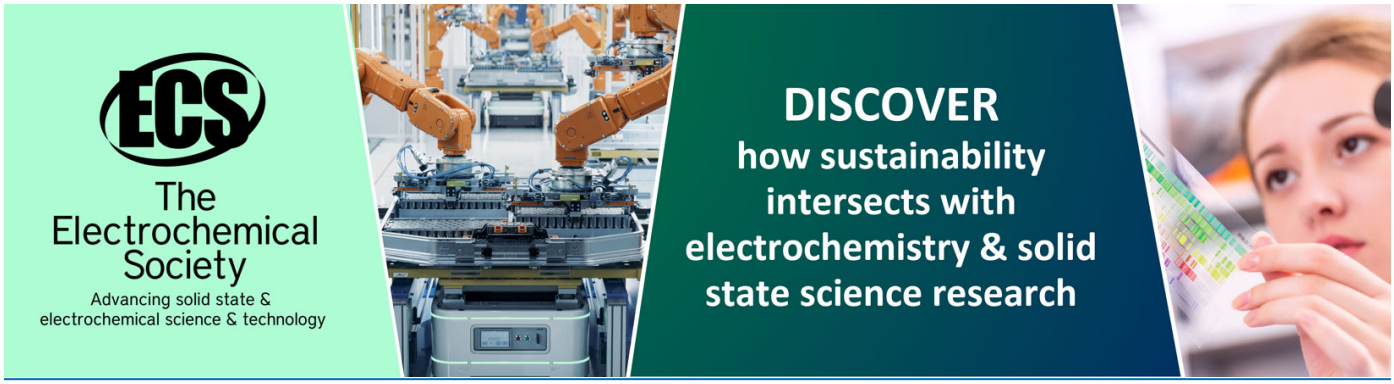
The effect of black cumin (*Nigella sativa*) extract on spermatozoa morphology of male albino rats (*Rattus norvegicus*) after induced with 2-Methoxyethanol



IOP Conf. Series: Journal of Physics: Conf. Series **1246** (2019) 012043 doi:10.1088/1742-6596/1246/1/012043

The effect of black cumin (*Nigella sativa*) extract on spermatozoa morphology of male albino rats (*Rattus norvegicus*) after induced with 2-Methoxyethanol

Indah Rahmadaniah[1*], Salni[2], Joko Marwoto[3], Sagita Darmasari[4]

[1] Abdurahman Midwifery Academy, Palembang, Indonesia
[2] Biology Department FMIPA, Universitas Sriwijaya, Palembang, Indonesia
[3] Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[4] Abdurahman Midwifery Academy, South Sumatra, Indonesia

E-mail : indahdaniah16@gmail.com

Abstract. The purpose of this study was to determine the effect of 2-ME and administration of black cumin extract on sperm motility of male white rats. The research method used a completely randomized design (CRD) of 24 male white rats. The 2-ME solution is given intraperitoneally, and black cumin extract is given orally. The samples were divided into 6 groups, treatment given for 21 days. Data analysis used One Way ANOVA and continued with Duncan's test. The results, after induced 2-ME spermatozoa morphology mean as much as 31.25%, after being given average black cumin extract spermatozoa morphology of 89.75%, after administration of 2-ME and black cumin extract dose of 1.2 g/kgBW average morphology of spermatozoa 35.75%, the dose of 2.4 g/kgBW mean morphology of spermatozoa was 47.00%, while the dose of 3.6 g/kgBW resulted in an increase in morphology of spermatozoa by 57.25%. Conclusion, 2-ME induction can reduce the morphology of male white rat spermatozoa. While the administration of black cumin extract can improve the morphology of male white rat spermatozoa. The administration of black cumin extract at a dose of 3.6 g/kgBW after 2-ME induced can improve the morphology of the sperm and is significantly different.

1. Introduction

In the world, the incidence of infertility is 50 million to 80 million couples or 8-12% of couples of childbearing age. Infertile couples of childbearing age are undergoing regular sexual intercourse without contraception for 12 months and did not experience pregnancy [1]. One of the causes of infertility that occurs in men is due to poor sperm quality that can be caused by environmental pollution, free radicals, and genetic disorders, while infertility in women is caused due to tubal disorders and ovulation [2].

Environmental pollution is one of the causes that can affect the male reproductive organs, many environmental pollutants that have the potential to interfere with the process of spermatogenesis and specifically give a high enough influence some of these materials include industrial materials, plasticizers, and metals [3]. Compounds of 2-Methoxyethanol (2-ME) are one of the metabolites of dimethoxyethilphtalate (DMEP). This DMEP is one group of phthalic acid ester (PAEs) which is widely used as a plasticizer in the manufacture of plastics [4].

In the body of a compound, 2-ME will spread widely and enter the blood circulation and then go to organs that are sensitive to these substances, namely the testes, spleen, and thymus [5]. MAA compound as a result of metabolism 2-ME compound is a strong oxidant and can cause oxidative

stress on sperm. Oxidation stress causes disruption in the phosphorylation oxidation process resulting in increased production of reactive oxygen species (ROS) spermatozoa. High ROS levels can oxidize lipids, proteins, and DNA. Lipid oxidation in the sperm membrane produces malondialdehyde (MDA) compounds, which are toxic to cells, causing spermatozoa damage. Damaged sperm membranes will cause a decrease in spermatozoa membrane integrity, which will cause a decrease in sperm quality [6]. 2-ME compounds can also cause a decrease in motility and morphology of spermatozoa [7].

To overcome the strong oxidation effects of 2-ME on spermatozoa, antioxidants that can counteract free radicals are needed. Many herbs that can be used as antioxidants include Black Cumin (Nigella Sativa). Thymoquinone contained in Nigella sativa has antioxidant activity which plays a very important role as a protector of spermatozoa against ROS [8].

Black cumin is a dicotyledonous plant from the Ranunculaceae family, this plant can be used as an extraordinary herb with a rich historical background and religion [9]. The chemical content of black cumin seeds is fat oil (fixed oil) (32% -40%), essential oil (0.4% -0.45%), protein (16% -19.9%), alkaloids, coumarin, minerals (1.79% -3.74%), carbohydrates (33.9%), fiber (5.5%), water (6%) [10]. The efficacy of this black cumin plant extract has been believed by the public to increase the quantity and quality of spermatozoa. This research was conducted to find out whether giving black cumin extract can improve the morphology of male white rats induced 2-ME.

2. Methods

This type of research is an experimental study with complete randomized design (CRD), which is a design with several treatments and arranged randomly for all experimental units. Black cumin seeds were obtained from the drug store as much as 1 kg than in a blender and then macerated with 96 liters of ethanol as much as 1 liter for 24 hours as much as 2x then filtered. After that, the evaporation process is carried out, the solution that has been left in is put into a flask, the evaporative flask is installed in the evaporator and the water bath is filled with water until it is fully heated and then adjusted to 900C and left until the ethanol solution separates with the active substance in the flask after it is continued to use a water bath. The black cumin extract obtained is then put into a glass and stored in the refrigerator until it is used, before using it needs to be left to stand so that the temperature is the same as the room temperature.

Tabel 1. Homogeneity of rat weight test results.

Group	**Treatment**	**Rat body weight (gram) mean ± SD**	***Levene Test (p value)***
K0	CMC 0,5% 1 ml	225,00 ± 05,774	
P1	2-ME 200mg/kg BB	230,00 ± 08,165	0,671
P2	Black cumin extract 1,2 g/kgBB	222,50 ± 12,583	
P3	2-ME 200mg/kgBB + Extract 1,2 g/kgBB	205,00 ± 10,000	
P4	2-ME 200mg/kgBB + Extract 2,4 g/kgBB	215.00 ± 05,774	
P5	2-ME 200mg/kgBB + Extract 3,6 g/kgBB	220.00 ± 14,142	

A sample of 24 rats was divided into 6 groups. The control group (K0) was given CMC 0.5% 1 ml orally 1x/day every morning for 21 days. Treatment group 1 (P1) was given 2-ME 200 mg/kgBW/day by intraperitoneal injection for 7 days and then given 0.5 ml/day of distilled water orally for 14 days. Treatment group 2 (P2) was given 0.5 ml/day of distilled water orally for 7 days and then given 1.2 g/kg/day of black cumin extract orally for 14 days. Treatment group 3 (P3) was given 2-ME 200 mg/kgBW/day intraperitoneally for 7 days and then given 1.2 g/kgBW/day of cumin extract orally for 14 days. Treatment group 4 (P4) was given 2-ME 200 mg/kgBW/day intraperitoneally for 7 days and then given 2.4 g/kgBW/day of cumin extract orally for 14 days. On the 22nd day after treatment, the rats were sacrificed, and the surgery was performed and the epididymis was taken. Spermatozoa were taken in the right part of the cauda epididymis, then cauda epididymis was placed in a petri dish containing 0.9 ml 1 ml NaCl and cut into small pieces and then left for 1-2 minutes to provide an opportunity for the spermatozoa to exit the epididymis and spread [11].

Sperm morphology observation was carried out by taking one drop of sperm from cauda epididymis then dripping on the slide and then drying and fixing with 40% methanol for 5 minutes

after rinsing with distilled water and drying. Next was given Giemsa dye for 15 minutes. After that, rinse again with tap water and aerated until dry. A total of 200 spermatozoa cells were observed in the shape of the head, neck abnormalities, tail abnormalities and cytoplasmic remnants using a light microscope with 400x magnification in each group. Then the results are expressed in percentage form [1]. Spermatozoa of normal rats have head shape features such as fishing hooks and long straight tails while abnormal spermatozoa have irregular head shapes (head shape like banana), amorphous head, double head, and tail, rolled tail, folded tail or broken tail [12].

3. Results

3.1. Sample Characteristics

The minimum body weight of rats used is 200 grams and a maximum of 240 grams. The average body weight of rats used in each group was 219.58 grams. From the results of rat body weight homogeneity test in table 1. above, it can be seen that the p-value = 0.671 (p> 0.05), which means the body weight of mice in each homogeneous treatment so that mice can be used in this study.

3.2. The Effect of Black Cumin Extract on Spermatozoa Morphology of Male White Rats in 2-ME Induction

The effect of black cumin extract on spermatozoa induced 2-ME was seen after the treatment of 24 rats, to see the comparison of the average spermatozoa morphology between groups was conducted One Way ANOVA test and continued with Duncan's further test obtained the following results.

Tabel 2. ANOVA test results continued duncan morphology test of spermatozoa (%) between group treatments.

Group	**Treatment**	**Spermatozoa Morphology (%) Mean ± SD**	***(P value)***
K0	CMC 0,5% 1 ml	84,25 ± 2,986 d	0,001
P1	2-ME 200mg/kg BB	31,25 ± 6,397 a	
P2	Black cumin extract 1,2 g/kgBB	89,75 ± 4,573 d	
P3	2-ME 200mg/kgBB + Extract 1,2 g/kgBB	35,75 ± 3,594 a	
P4	2-ME 200mg/kgBB + Extract 2,4 g/kgBB	47,00 ± 3,916 b	
P5	2-ME 200mg/kgBB + Extract 3,6 g/kgBB	57,25 ± 3,594 c	

Description: The numbers followed by the same lowercase letters are not significantly different at a further test of Duncan (p<0,05).

4. Discussions

Table 2 shows the ANOVA test results p = 0.001 (p <0.05), so it can be concluded that the extract of black cumin has the influence to improve the morphology of normal sperm and 2-ME induction can damage the morphology of normal spermatozoa. To see the significance between groups, Duncan continued the test, which showed that there was a decrease in the normal morphology of spermatozoa in group P1 which was significantly different from the K0 group. In the P3 group, the morphology of normal spermatozoa decreased and was not significantly different from the P1 group. In group P4, it can be seen that there is a significant difference in the normal morphology of spermatozoa compared to groups P1 and P3 but cannot match the group K0. Whereas in group P5 it can be seen that there is a significant difference where the morphology of normal spermatozoa is more than the group P1, P3, and P4 but still significantly different from the group K0.

In figure 1 it can be seen the difference in normal sperm morphology between treatment groups. The morphological decline in normal spermatozoa occurred in group P1 and after being given a different treatment there was an increase in P3, P4 and P5 groups. Of the three groups, the increase cannot be equal to the K0 and P2 groups, so the graph difference is very far away.

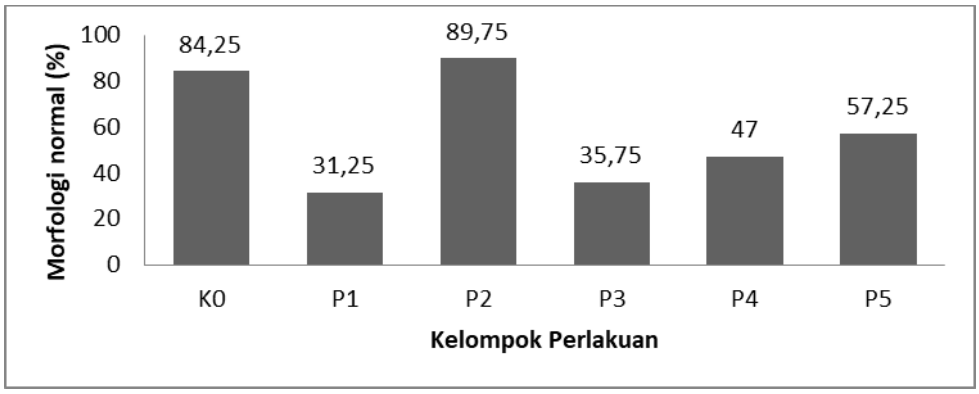


Figure 1. Spermatozoa morphology charts between treatment groups.

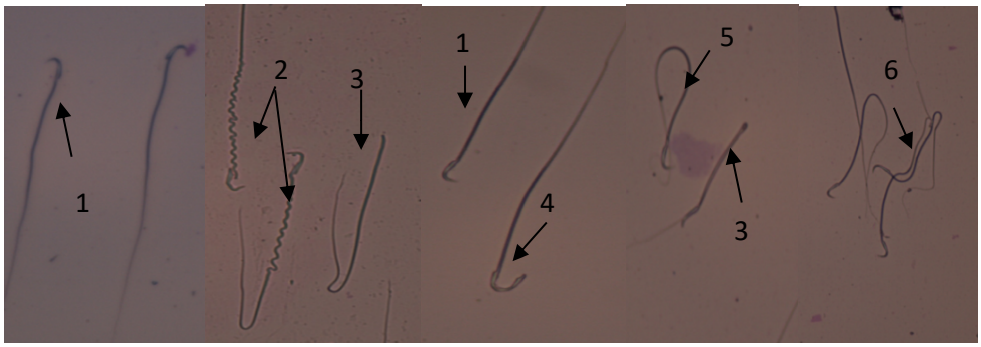


Figure 2. Morphology of spermatozoa (1) normal spermatozoa (2) jagged neck (3) broken head (4) large head (5) rolled tail (6) folded tail.

The intraperitoneal administration of 2-ME at a dose of 200 mg/kg body weight in this study has been shown to reduce the morphology of normal spermatozoa to 31.25%. This is due to the increased production of ROS, which causes the epididymis to be a place of disrupted sperm maturation and results in damage to the formation of spermatozoa. Spermatozoa deformities can also be caused by excess Ca2 + so activating protease and phospholipase enzymes that are able to degrade cytoskeletal proteins are needed in building cellular structures [13]. Degradation of cytoskeletal proteins is one of the causes of damage in the cellular process, the formation of structures, resulting in spermatozoa morphological abnormalities. 2-ME also disrupts the lactate production of Sertoli cells. As a result, the development of spermatocytes, which require lactate as an energy substrate, is also disrupted, causing abnormal morphology of spermatozoa [14].

In this study the forms of abnormalities found due to 2-ME induction are, deformities in the neck that are jagged, tail rolled, head broken and head large. This disorder is caused because ROS can increase lipid peroxidation and increase the permeability of cell membranes to produce hydrogen peroxide. This hydrogen ion reacts with unsaturated fatty acids which will produce new hydrogen peroxide and lipid radicals. Decomposition of these unsaturated fatty acids to form fragments of low molecular weight fatty acids one of which is Malondialdehyde (MDA). MDA compounds are used as indicators of lipid peroxidation in membrane cells that cause damage to cell membrane structure [15]. Spermatozoa membrane damage can affect sperm morphology which can cause a decrease in the quality of other spermatozoa such as motility and viability of sperm [16].

The levels of oxygen reactive compounds or free radicals can be balanced in the body, but if the number of ROS compounds is more than the levels of antioxidants, this condition is called oxidative stress. To overcome this, adequate antioxidants are needed to be able to counteract free radicals so that they can protect cells from damage. In this study, antioxidants were obtained from black cumin extract.

In this study, the administration of black cumin extract with a dose of 1.2 g/kgBB after 2-ME induction has shown a slight increase in the normal spermatozoa morphology of 35.75% but not

significantly different than the group that was only given 2-ME 200 mg/kgBB which is 31, 25%. While the black cumin extract with a dose of 2.4 g/kgBB showed an increase in the morphology of normal spermatozoa with a mean of 47% greater than the group P1 which was only given 2-ME. Whereas in the treatment group which was given 3.6 g/kg BB black cumin extract the average morphology of normal spermatozoa was higher at 57.25% compared to the group given only 2-ME and the group has given 2-ME + cumin extract1,2 and 2,4 g/kgBB. Morphological improvement can also be seen in research conducted by Maruliyananda et al [17], with the results of giving an ethanolic extract of mung bean sprouts at a dose of 0.5, 1 and 2 g/kg body weight can increase the number, and normal morphology of exposed mice spermatozoa 2 -ME. Whereas according to research conducted by Hayati et al [7] showed a decrease in the morphological percentage of normal spermatozoa to 40.28% and after exposure to 2-ME 200 mg/kgBB intraperitoneally for 3 weeks and termination for 1 week. Then it increased to 65.16% after exposure to 2-ME for 3 weeks and stop for 4 weeks. The normal morphology of spermatozoa can be recovered despite not being given any treatment within 4 weeks.

5. Conclusions

2-ME induction 200 mg/kgBB can affect the spermatozoa maturation process, thereby reducing the morphology of spermatozoa, male white rats. Black cumin extract can improve the spermatozoa maturation process so as to improve the morphology of male white rat spermatozoa. The provision of black cumin extract at a dose of 3.6 g /kgBW after induced 2-ME has been able to improve the morphology of spermatozoa and has been significantly diff.

6. References

[1] WHO and Edition F 2010 Examination and processing of human semen *World Heal. Ed.* **10** 286
[2] Speroff L and Fritz M A 1999 Clinical Gynecologic Endocrinology and Fertility
[3] Perreault . S.D 2014 *Efek Keterpaparan Zat Kimia Terhadap Pria, Reactive Oxygen Species dan Spermatozoa, Hanbook of Andrology* ed N Arsyad, K.M dan Moeloek (Palembang. Musigrafika)
[4] Butterworth M, Creasy D and Timbrell J A 1995 The detection of subchronic testicular damage using urinary creatine: studies with 2-methoxyethanol *Arch. Toxicol.* **69** 209–11
[5] Miller R R, Hermann E A, Langvardt P W, McKenna M J and Schwetz B A 1983 Comparative metabolism and disposition of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats *Toxicol. Appl. Pharmacol.* **67** 229–37
[6] Hayati A, Mangkoewidjojo S, Hinting A and Moeljopawiro S 2006 Hubungan Kadar Mda Sperma Dengan Integritas Membran Spermatozoa Tikus (Rattus Norvegicus) Setelah Pemaparan 2–Methoxyethanol *Berk. Penel. Hayati* **11** 151–4
[7] Hayati A, Rahmaninta A D and Pidada I B R 2005 Spermatozoa Motility and Morphological Recovery Process in Mice (Mus musculus) after the Induction of 2-Methoxyethanol *Folia Medica Indones.* **41** 90
[8] Khotimah S 2006 Pengaruh pemberian ekstrak jinten hitam (Nigella sativa) terhadap kadar GHS paru dan hepar tikus Wistar yang dipapar asap rokok *J. Biosains Pascasarj.* **8** 7–12
[9] Goreja W G 2003 *Black seed: nature's miracle remedy* (Amazing Herbs Press New York)
[10] Randhawa M A and Al-Ghamdi M S 2002 A review of the pharmaco-therapeutic effects of Nigella sativa *Pak J Med Res* **41** 77–83
[11] Simbolon I S, Lubis T M and Adam M 2013 Persentase spermatozoa hidup pada tikus wistar dan sprague-dawley *J. Med. Vet.* **7**
[12] Nita S 1998 *Pengaruh Mangostin Terhadap Kualitas Sperma Epididimis Cauda Tikus Wistar Jantan,* (Institute Teknologi Bandung)
[13] LI L, WINE R N and CHAPIN R E 1996 2-Methoxyacetic acid (MAA)-induced spermatocyte apoptosis in human and rat testes: an in vitro comparison *J. Androl.* **17** 538–49
[14] Gray T J B, Moss E J, Creasy D M and Gangolli S D 1985 Studies on the toxicity of some glycol ethers and alkoxyacetic acids in primary testicular cell cultures *Toxicol. Appl. Pharmacol.* **79** 490–501
[15] Kim B-S and Smialowicz R J 1997 The role of metabolism in 2-methoxyethanol-induced

suppression of in vitro polyclonal antibody responses by rat and mouse lymphocytes1 *Toxicology* **123** 227–39

[16] Panghiyangani R and Mashuri 2009 Kualitas Spermatozoa dan Aktivitas Enzim Katalase dalam Darah Tikus Jantan Galur Sprague Dawley (SD) yang Diradiasi Sinar Ultraviolet *J. Kedokt. Indones.* **1** 4–7

[17] Maruliyanada C, Hayati A and Pidada I B R 2012 Pengaruh Ekstrak Etanolik Kecambah Kacang Hijau (Phaseolus radiatus) terhadap Jumlah dan Morfologi Spermatozoa Mencit yang Terpapar 2-Methoxyethanol *Artik. ilmiah. Progr. Stud. Biol. Dep. Biol. Fak. Sains dan Teknol. Univ. Airlangga. Surabaya* 23–5