The effectiveness of the *Macrotermes gilvus* termite queen for sperm repair in infertile mice

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**Abstract.** Handling infertility health problems can be done by improving sperm quality. This study aims to analyze the effectiveness of *Macrotermes gilvus* termite queen in improving sperm quality in infertile mice due to lead contamination. This study used a post-test only control group design with 6 groups, each group contain 4 termites. Test animals were given lead acetate of 8 mg / kg BW orally for 14 days, then the treatment group was given the termite queen *Macrotermes gilvus*, respectively 50, 100, and 200 mg / kg BW for 21 days. Test animals were dissected, then sperm were taken to be analyzed for quality based on concentration, motility, morphology, and sperm viability. Data were analyzed using One-way ANOVA with LSD test. The results showed the termite queen *M. gilvus* was effective in repairing sperm based on concentration, motility, morphology, and viability. However, lead did not succeed in damaging the morphology of the sperm so that the administration of the termite queen *M. gilvus* did not have a significant effect. The termite queen *M. gilvus* can be an alternative ingredient to improve sperm quality.

1. Introduction

Infertility health problems are important to be addressed because there is an increase in cases each year. In 2013, infertile couples in Indonesia reached 15-25% [1]. About 20 - 70% of infertile cases are caused by male factors [2]. Abnormal sperm quality is owned by 33% of infertile men [3]. Sperm abnormality can be caused by an imbalance between Reactive Oxygen Species (ROS) and antioxidants in the body or known as oxidative stress. Under normal conditions, oxidative stress plays a role in the physiological function of reproduction. However, excessive oxidative stress can spur pathological processes in the reproductive tract, including infertility [4]. ROS levels in sperm from infertile men are 25% higher than fertile men. Therefore, it is very interesting to handle infertility cases by reducing excess ROS in the body in infertile men. One of them is the addition of antioxidants from outside the body [5].

Antioxidants are available in natural materials, one of which is the termite queen *Macrotermes gilvus* which is commonly consumed by the public. The termite nests of *M. gilvus* are widespread in Indonesia [6]. One of the ingredients in the termite queen *M. gilvus* is methionine which can act as an antioxidant and a precursor for endogenous antioxidant production. The level of methionine from termite queen *M. gilvus* which had gone through the freeze-drying process was 11.05 mg / g [7]. The body really needs these essential amino acids. However, the body cannot produce it alone. Therefore, additional intake is needed from outside the body, one of them from the termite queen. This study aims to analyze the effectiveness of the termite queen *M. gilvus* on sperm repair in infertile mice due to the administration of lead acetate.
2. Methods
The research design used was a post-test only control group design. The total test animals were 24 male mice (*Mus musculus*) with age criteria 8-12 weeks and weight 20-30 grams. Test animals were obtained from a mouse farm in Tembalang, Semarang. The independent variable is the dose of the termite queen *M. gilvus* (50, 100, and 200 mg / kg BW). The dependent variable is the sperm quality of the test animals (concentration, motility, morphology and viability).

2.1. *M. gilvus* sample making
The termite queen *M. gilvus* was obtained from a garden on the campus of State University of Semarang in Sekaran. The termite queen is then processed for freeze-drying for 3 days at the Food Technology Laboratory at Soegijapranata Catholic University, Semarang. The dry termite queen is then mashed using mortar and pestle until it becomes a homogeneous powder. The sample is put into a dry flakon bottle and tightly closed, then stored at 4 °C for further analysis.

2.2. Treatment of test animals
Test animals acclimatized for 7 days, then treated according to the group, ie 1) normal control, without treatment; 2) negative control by giving lead acetate 8 mg / kg BW for 14 days; 3) normal treatment with 8 mg / kg BW of lead acetate for 14 days, then not treated for 21 days; 4) the treatment of dose I by giving lead acetate 8 mg / kg BW for 14 days, then continued with the administration of the termite queen *M. gilvus* 50 mg / kg for 21 days; 5) the treatment of dose II by giving lead acetate 8 mg / kg BW for 14 days, then continued with the administration of the termite queen *M. gilvus* 100 mg / kg BW for 21 days; and 6) treatment of dose III by administration of lead acetate 8 mg / kg BW for 14 days, then continued with the administration of the termite queen *M. gilvus* 200 mg / kg for 21 days. All test animals were fed and drank *ad libitum*.

2.3. Sperm sample collection
Test animals were anesthetized using chloroform cotton, then the test animals were dissected and their vas deferens were taken. The vas deferens are placed in a Petri dish which has been given a 0.5 ml physiological NaCl solution for each test animal. Sperm in the vas deferens is removed by sorting the vas deferens using a surgical instrument. The sperm that has been released is then homogenized with physiological NaCl as a sperm stock solution.

2.4. Sperm quality analysis
2.4.1. Sperm concentration. Sperm concentration is calculated based on the number of sperm observed in the Improved Neubauer haemocytometer booth. Sperm is diluted 20 times first using an erythrocyte pipette, then dropped on a hemocytometer. Observation is made using a 40x magnification microscope. The calculation of sperm for dilution 20 times is 106 / ml, then the concentration of sperm is expressed using the following formula:

\[ K = N \times 10^6 / ml \]

2.4.2. Sperm Motility. A sperm stock solution of 10-15 μl is dripped on a clean, fat-free glass. Sperm motility was observed using a microscope with a magnification of 10x in three fields of view, then estimated the percentage of each category of sperm motility according to the standard [8], ie progressive if the sperm moves straight forward, non-progressive if the sperm moves rotating, and immotile if the sperm do not move. The percentages of the three fields of view are then averaged.

2.4.3. Sperm morphology and viability. A 10 μl sperm stock solution is placed on a clean, fat-free glass of another object. Methanol fixative is dripped evenly on all parts of the sperm smear, then air-dried. After drying, the eosin dye is dripped evenly on all parts of the sperm smear, then dried. After drying, the preparation is washed with running water so that it is clean from the dye, then re-dried. Preparations were observed using a microscope with a magnification of 40x. Sperm morphology is classified based on normal and abnormal criteria, then expressed in
percent. Sperm viability is calculated based on the number of live and dead sperm. Live sperm will appear transparent, while dead sperm will be red in the head. Sperm viability is expressed in percent using the formula:

\[
\text{Viability} = \frac{\text{number of live sperm}}{\text{total sperm live and die}} \times 100\%
\]

### 2.5. Data analysis

Statistical analysis of sperm quality data is performed using One way ANOVA. If there are differences, then LSD further tests. The application used is SPSS 16.0.

### 3. Results and Discussion

The results of this study are the sperm quality of test animals with parameters of concentration, motility, morphology, and viability (see Table 1). The normality test showed data of concentration, motility, morphology, and sperm viability were normally distributed (p> 0.05). Sig value mean concentration, motility, and sperm viability of p <0.05 then H0 is rejected. So, the mean concentration, motility, and viability of sperm in each group are not the same. While the value of sig. mean normal morphology of p> 0.05 then H0 is accepted. So, the normal morphology of sperm in each group is the same.

#### Table 1. Average concentration, motility, morphology, and sperm viability.

| Group | Sperm Concentration (10^6/ml) | Imotile Sperm Motility (%) | Normal Morfolgy (%) | Sperm Viability (%) |
|-------|-----------------------------|---------------------------|---------------------|-------------------|
| 1     | 22,75^a                      | 19,17^a                   | 93,75^a             | 91,25^a           |
| 2     | 10,25^b                      | 27,50^b                   | 73,50^a             | 1,25^b            |
| 3     | 15,50^c                      | 27,08^c                   | 90,75^a             | 4,00^b            |
| 4     | 15,75^ce                     | 23,75^b                   | 91,00^a             | 23,00^b           |
| 5     | 16,67^abc                    | 23,33^b                   | 92,75^a             | 43,75^b           |
| 6     | 19,50^abc                    | 23,33^ab                  | 93,75^a             | 46,33^ab          |
| Sig.  | 0,025                        | 0,007                     | 0,76                | 0,000             |

Note: the number followed by the letters in the same column shows the difference in each treatment group with a level of accuracy p <0.05.

Based on statistical analysis, the administration of the termite queen *M. gilvus* affected the concentration, motility, and viability of sperm, but did not affect the morphology of the sperm. Groups 5 and 6 have the same letters as group 1 on the average sperm concentration and motility. That is, the administration of the termite queen *M. gilvus* 100 mg / kg BW and 200 mg / kg BW can improve sperm quality based on sperm concentration and motility parameters. Based on normal morphological parameters, all groups have the same letter. That is, giving lead acetate 8 mg / kg BW for 14 days does not damage the morphology of the sperm and the administration of the termite queen *M. gilvus* has no effect on sperm morphology. Based on the parameters of sperm viability, group 6 has the same letters as group 1. That is, giving termites queen *M. gilvus* 200 mg / kg BW can improve sperm quality based on sperm viability parameters.

Group 2 by giving lead 8 mg / kg BW for 14 days succeeded in reducing sperm quality based on concentration, motility, and viability. This is in accordance with previous studies which reported that lead acetate 8 mg / kg BW given to rats for 14 days can damage testicular histology, decrease sperm count and viability compared to the control group [9]. However, lead administration in this study had no effect on sperm morphological abnormalities. This is expected because the duration of lead in this study is too short, so it does not cause sperm morphological damage related to DNA damage and chromatin structure. More time is needed to damage the sperm morphology, according to studies
reporting that administration of drinks with 0.5% and 1% lead concentrations in mice for 6 weeks causes an increase in abnormal morphology, DNA damage and damage to chromatin structure [10].

Lead affects male reproduction through several mechanisms. After lead is absorbed through the digestive tract, lead enters the bloodstream into the tissues. In the blood, lead inhibits deltaaminolevulinic acid dehydratase (ALAD), a heme synthesis enzyme. As a result, an accumulation of delta aminolevulinic acid (ALA) can produce oxidants. The ability of oxidants to bind to the -SH groups of reduced glutathione (GSH) and oxidized glutathione (GSSG) disrupts the balance of GSH / GSSG, so cells experience oxidative damage. Although the body has antioxidants to protect cells from free radicals, lead has the ability to increase ROS while antioxidants continue to decrease. Oxidation of lipid membranes due to cell interactions with lead causes changes in integrity, permeability and membrane function [11].

In addition to activating ALAD, lead also activates glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase which causes a decrease in the level of glutathione in the body. Antioxidant enzymes that can be inactivated by lead include SOD and CAT. A decrease in SOD levels can reduce the removal of superoxide radicals, whereas a decrease in CAT disrupts the clearance of superoxide radicals (O2-). Free radicals produced from lead cause lipid peroxidation because it can capture electrons in the lipids from the cell membrane to further cause cell damage [12]. Provision of 0.3% lead acetate solution to rats for 90 days showed an increase in lead concentration and a decrease in zinc concentration as well as a significant decrease in sperm parameters (vitality, motility, density, and sperm morphology). Zinc in the testes is an indicator of a balance between antioxidants and pro-oxidants [13]. Lead of 350 mg / kg BW given to mice decreased CAT activity and increased lipid peroxidation [14].

Lead exposed to mice can inhibit the production of GnRH, so that the production of FSH and LH by the pituitary gland is inhibited and testosterone production is inhibited. This causes inhibition of spermatogenesis and sperm produced are not optimal [15]. Other studies also mention that men exposed to lead in China show that higher lead concentrations cause a decrease in the concentration of pituitary hormones (FSH, and LH) as well as a decrease in testosterone which is a steroid hormone produced by Leydig cells with stimulation from the LH hormone [16].

Provision of queen termite M. gilvus can increase sperm concentration, motility, and viability. One of the contents in the termite queen M. gilvus is methionine as a source of oxidants. Other studies report that administration of methionine at a dose of 0.5% can increase spermatogenesis and epididymis histopathology of mice (17), addition of methionine (1.5 mM) in carp sperm (Carassius auratus) significantly influence the increase in sperm motility and decrease DNA damage [18], and an increase in the integrity of the acrosome and plasma membrane of Merino sheep sperm by giving methionine (1 mM). In addition, methionine has a positive role to improve the reproductive performance of broilers with higher fertility, hatching, and birth parameters than in the control group. This is because glutathione and cysteine as a result of methionine metabolism can sweep ROS directly, so that the detrimental effects of ROS on lipids, proteins, and DNA can be reduced [19].

Methionine can reduce oxidative peroxidation by activating the formation of endogenous antioxidants [20]. Methionine from the termite queen M. gilvus can protect sperm cells from oxidative damage due to lead because it can react directly with oxidants and become a precursor for glutathione production, resulting in inactivation of ROS (4). Endogenous antioxidant glutathione plays an important role in suppressing lipid peroxidation and inactivation of ROS through the redox cycle [21].

Sperm concentration and viability can be increased after administration of methionine from the termite queen M. gilvus because the sperm membrane is protected from damage to lipid peroxidation caused by free radicals from lead generation. Methionine reacts quickly with mild oxidants to form methionine sulfoxide (MetO) and reacts quickly with stronger oxidants to form methionine sulfone (MetO2) [22]. The results of this reaction are stable and not destructive. Besides directly acting as an antioxidant, methionine is also a precursor for the production of endogenous antioxidants, both glutathione and antioxidant enzymes such as GSH-Px, GST, and SOD [23]. Increased redox potential of glutathione and activation of these antioxidant enzymes can increase fertility potential [24].
Sperm is produced through several stages in spermatogenesis. If the concentration of spermatogonia decreases since the beginning of spermatogenesis, the concentration of sperm produced will decrease. If sperm during the process of spermatogenesis or sperm are ripe with oxidative peroxidation, then the survival (viability) of sperm produced will be reduced. Lipid peroxidation from the sperm membrane is a key mechanism of sperm damage. Therefore, the protection of methionine against lipid peroxidation is able to maintain the sperm membrane from oxidative damage, so that sperm concentration and viability can be increased. Progressive motility is reduced due to ROS administration because electrons from ROS are added directly to the protein that functions to regulate sperm motions [25]. Protein from the termite queen *M. gilvus* can increase sperm metabolism, that metabolic protein with unique expression in sperm can correct adaptive changes to increase the efficiency of ATP production and a higher energy supply for motility [26].

4. Conclusion

Based on the above discussion it can be concluded that the termite queen *M. gilvus* can improve sperm quality in infertile mice due to lead administration, with parameters of sperm concentration, motility, and viability. However, the administration of lead did not cause sperm morphological damage, so the administration of the termite queen *M. gilvus* had no effect on sperm morphology. The termite queen *M. gilvus* can be used as a source of methionine for the solution to infertility health problems.

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