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ORIGINAL ARTICLE

Protective Effect of Lemongrass Extract (*Cymbopogon nardus*) on Spermatozoa Cells of Lead Acetate Induced Mice

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ABSTRACT

Introduction: Male infertility can be caused by many factors. One of which is environmental pollution such as lead acetate. Lead acetate exposure can increase Reactive Oxygen Species (ROS), affecting spermatozoa quality. Natural antioxidants and flavonoid on Lemongrass (*C. nardus*) have a great potential for protecting the male reproductive system. This study aimed to analyze the effect of giving various dosages of *C. nardus* extract on the sperm quality of mice.
Methods: The Balb/C male mice were divided into five equal groups. The negative control group was injected with 0.01 ml Na-CMC 0.5% within 40 days, and the positive control group was injected with 0.01 ml lead acetate 7 mg/kg BW within 5 days, the treatment groups were injected by 0.01 ml lead acetate 7 mg/kg body weight within 5 days and continuously injected by 0.01 ml of *C. nardus* extract with various dosage 25 mg/kg BW, 50 mg/kg BW, and 100 mg/kg BW within 35 days. At the end of the experiment, mice were sacrificed, and sperm suspensions were collected from cauda epididymal to measure the morphology, concentration, and motility.
Results: The results showed that giving *C. nardus* extract could repair morphology, concentration, and motility of spermatozoa with significantly different ($p<0.05$). The 25 mg/kg BW dose has a good protective effect.
Conclusion: The *C. nardus* extract can repair the decreasing sperm quality caused by lead acetate exposure.

Introduction

The prevalence of infertility in Indonesia is increasing every year. Infertility does not only occur in womenbut also in men. Men are responsible for 36% determined fertility success.[1] Many factors can cause male infertility, one of them is environmental pollution such as lead acetate. Lead acetate is usually used for battery manufacture, cable coating, and leaded gasoline. The waste of lead acetate has a potential effect on sperm quality.[2] Lead is a toxic metal that can affect manyorgans and systems in the human body and can induce an increase in Reactive Oxygen Species (ROS) through a lipid reaction that produces lipid peroxide.[3,4]

Many studies revealed that lead acetate could cause infertility in male.[5] A study revealed that giving 0.1% lead acetate to rats had reduced testis weight, seminiferous tubulous diameter, epididymal sperm count, and serum testosterone level.[6] Another study revealed that 20 mg/kg BW lead acetate could caused the decrease of testis weight and diameter of tubulous seminiferous in male mice testis.[7] Lead acetate disturbed the antioxidant enzyme activities with bind sulfhydryl bonds, which increased oxidant level.[8] Thus, treatment with antioxidant compounds could inhibit the adverse effects of lead acetate on the reproductive system.[9,10] In this case, the use of certain plants containing antioxidant is considered to reduce the toxic effect of various toxicants including lead acetate. One of the natural antioxidants with plenty of benefits and commonly used as herbal medicine is of lemongrass. In this research, *C. nardus*, one kind lemongrass, is known for its antioxidant activity and effective scavenging mechanisms for free radicals through its potent flavonoid compound.[10,11] Therefore, this research investigated the potential protective of *C. nardus* extract on the male reproductive system

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after being induced by lead acetate. The parameters of measurement were morphology, concentration, and motility of spermatozoa.

Methods

Ethical clearance

All procedures in this study including using mice as the animal model had been approved by the Ethical Review Committee, Research and Community Service Department of Universitas Brawijaya, East Java, Indonesia with the number 120-KEP-UB-2021.

Plant materials

The *C. nardus* was collected from Materia Medica, Batu, East Java in August 2021. It was recognized and authenticated in the Biological Laboratory, Biology Department of Universitas Muhammadiyah Lamongan.

Preparation of 0.5% Na-CMC suspension

Five hundred milligrams of Na-CMC were weighed and then dissolved in some warm distilled water. After dissolving, all the remaining distilled water was added to obtain a 100 ml Na-CMC solution volume using a 100 ml measuring flask.

Preparation of ethanolic extract of *C. nardus* and suspension

The stalks of *C. nardus* were cut into small parts and dried under the sun for approximately five days. The dried stalks (250 grams) were powdered in an electric grinder and maceration with ethanol within 3 days at room temperature. Then, the extract was filtered and concentrated by heating in a water bath at a temperature of 70°C. To make suspension, the extract was homogenously crushed with 0.5% Na-CMC. The concentrations were 0,5%, 1%, and 2%

Preparation of Lead acetate suspension

For five days of stock, seven milligrams of lead acetate were weighed, and then dissolved in some warm distilled water. After dissolving, all the remaining distilled water was added to obtain a volume of 15 ml Na-CMC.

Chemicals and Reagent

Ethanol, methanol, lead acetate, eosin, nigrosine, and chloroform (purchased from Merck, Darmstadt, Germany), distilled water, and NaCl 0.9%.

Animals

Thirty adult male Balb/C mice (8 weeks old, 25–30 gram body weight) were obtained from the Veterinary and Pharma Center, Surabaya, East java. They were housed under standard laboratory conditions (temperature 28-30°C, 12-h/12-h light/dark cycle) and given food and water ad libitum.

Experimental design

After one week of acclimatization, animals were divided randomly into five equal groups (n=6) as follows: the negative control group were given a subcutaneous injection of 0.1 ml Na-CMC 0.5% within 40 days, positive control group were given a subcutaneous injection of 0.1 ml lead acetate with a dosage of 7 mg/kg BW within 5 days. The treatment group was given a subcutaneous injection of 0.1 ml lead acetate with a 7 mg/kg body weight dosage within 5 days. Then, the subcutaneous injections of 0.1 ml with various dosages were given in each treatment group. The first treatment group was 25 mg/kg BW, the second treatment was 50 mg/kg BW, and the third treatment was 100 mg/kg BW within 35 days. After the end of the procedure, all mice were killed using chloroform. Then, cauda epididymal was collected and made a sperm suspension for sperm analysis measurement.

Sperm analysis

In this study, a suspension of spermatozoa was used for measuring motility, morphology, and concentration. For analysis of sperm motility, 10µL was placed in a hemocytometer chamber and analyzed under a light microscope. One hundred spermatozoa were evaluated per animal and classified into motile and immotile. Motile is spermatozoa which have a straight and progressive movement. For evaluation of sperm concentration, an hour after the sperm diffusion in the solution, 10µL of the sperm suspension was transferred to each counting chamber of the hemocytometer and left for 5 minutes. Then, sperm heads were counted by a light microscope at 40 magnification and expressed as million/ml of suspension. The sperm morphology was also determined using the eosin-nigrosine staining method. For this purpose, suspension of spermatozoa was dropped on top of the object's glass to make smear preparations and dried in the air. The smear preparations were fixed with methanol, colored with 1% Y eosin solution and nigrosine, and left to dry. The preparations were washed with distilled water and dried. The preparation was observed under a light microscope with a magnification of 400 times to determine the morphology of 100 spermatozoa of mice. The last step was to calculate the percentage of normal and abnormal spermatozoa.

Results

The results showed that lead acetate administration to mice after 5 days led to a significant ($p<0.05$) decrease in sperm concentration and percent of motile spermatozoa. The percentage of spermatozoa with abnormal morphology was statistically ($p<0.05$) higher in lead acetate induced mice compared to a negative control group. The sperm parameter increased significantly ($p<0.05$) in mice administered by *C. nardus* extract compared to mice induced by lead acetate (Table 1).

The data in the table show that spermatozoa motility and concentration with a dose of 100 mg/kg BW of *C. nardus* extract were not significantly different from the positive control group. This means that it was unable to restore the quality of spermatozoa damaged by high doses of lead acetate. Furthermore, at a dose of 25 mg/kg BW of *C. nardus* extract showed the highest average in observation parameters. It can be assumed that the dose was optimal in improving the quality of spermatozoa. Description of sperm morphological observation is shown in Figure 1 and the differences of each group on sperm morphology in Figure 2.

Table 1. Effect of extract on sperm parameters in lead acetate induced mice.

Parameters	Negative control (Na-CMC)	Positive control (Lead acetate)	Lead acetate + *C. nardus* 25 mg/kg bw	Lead acetate + *C. nardus* 50 mg/ kg bw	Lead acetate + *C. nardus* 100 mg/ kg bw
Motility	56.33 ± 4.76	32.83 ± 5.57a	51.17 ± 3.67b	42.33 ± 2.94b	38.33 ± 7.23a
Normal morphology	87.83 ± 2.32	26.33 ± 2.73a	79.67 ± 3.56b	61.33 ± 3.14b	48.17 ± 4.71b
Concentration	46.17 ± 5.85	27.50 ± 3.39a	44.33 ± 4.18b	41.17 ± 5.60b	33.83 ± 5.42a

Data are presented as mean ± SD

a Significantly different ($P < 0.05$) between positive control group and negative control group (in rows).

b Significantly different ($P < 0.05$) between C. nardus treatment group and positive control group (in rows).

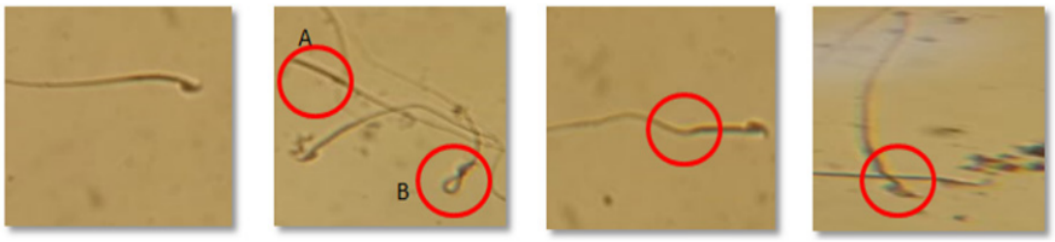

Figure 1. Spermatozoa morphology in adult mice with normal (N) and abnormal (A: head malformation (like needle); B: bent midpiece or tail; C: broken tail; bigger head)

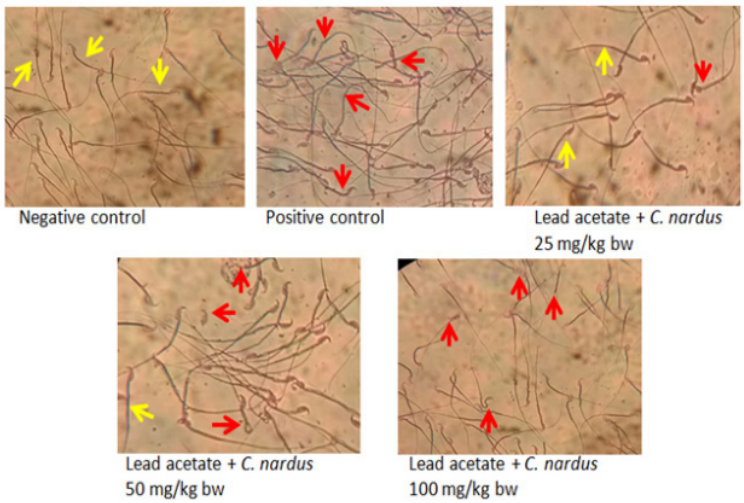

Figure 2. The differences of each group on sperm morphology (: normal sperm; : abnormal sperm

Discussion

According to the results, *C. nardus* extract had effect on restoring the decreased quality of spermatozoa due to lead acetate exposure. There was a significant difference ($p<0,05$) between the group administered with *C. nardus* extract and the positive control group treated with lead acetate.The 25 mg/kg BW dose was the optimal dose to improve morphology, motility, and concentration of spermatozoa. The higher dose given did not result in an increase in the spermatozoa quality as seen in 100 mg/kg BW dose could not improve sperm quality after being treated by lead acetate.

Lead is known as a metal that can be used in several materials but has a toxic effect on the human and animal organs.[12] Acute toxicity of lead acetate rarely occurs, but after a long period of exposure it can cause chronic effects, accumulated in the blood, and cause some disorders.[13] The lead toxicity mechanism can be summarized as three pathways. The first is blocked hypothalamic-pituitary testicular which causes a lower level of LH (Luteinizing Hormone) caused by inhibition of GnRH. The second is increasing ROS. The third is decreasing antioxidant activity by inactivating glutathione by binding to its sulfhydryl parts.[13,14]

Lead acetate that affects cell damage is indicated by a decrease in the number of spermatogenic cells, leydig cells, and sertoli cells.[15]

The study described that induced 75 mg/kg body weight in mice decreased the testosterone level, number of leydig cells, and spermatogenic cells.[16] This is in line with previous study which revealed that inducing 7 mg/kg body weight in mice decreased the spermatozoa quality such as the concentration, motility, and normal morphology. Sperm membranes contain high concentration of poly-unsaturated lipids so that making them sensitive to ROS and oxidative damage.[17] Lipid peroxidation can cause impaired motility, abnormal morphology, reduced sperm-oocyte fusion, and sperm cell death.[18,19] Arecent study shows that *C. nardus* is one of the herbal medicines that potentially protect organsfrom free radicals. The phytochemical compounds in *C. nardus* are antioxidant, flavonoid, phenolic, and terpenoids.[20,21] Antioxidants protect lipids membrane by sweeping superoxide ammonium and peroxide which can trigger cell damage and play a role in electron transport in the end stage of energy formation in mitochondria. The formation of ATP or energy by mitochondria is needed to increase sperm motility in fertilitazion.[22,23] A Previous study has described the protective effect of lemongrass in male reproductive after induced by hydrogen peroxide. This study shows that 100 mg/kg body weight of lemongrass extract can increase testosterone level, sperm characteristics, testicular and epididymal weight, and decrease serum and tissue homogenate MDA and testicular histopathological.[10] In this study, the *C. nardus* extract could increase sperm quality in mice after being induced by lead acetate and the potential dose which could increase sperm quality significantly was *C. nardus* 25 mg/kg BW.

Conclusion

The *C. nardus* extract has a protective effect on spermatozoa cells by repairing the decreasing osperm quality caused by lead acetate exposure. A 25 mg/kg BW dose has a more sufficient protective effect. A high dose of *C. nardus* extract is not able to repair sperm motility and concentration, but it can repair abnormal morphology.

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Conflict of Interest

The Authors declares that there is no conflict of interest.

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