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RESEARCH ARTICLE

Examining the Effects of Lemongrass (*Cymbopogon nardus*) Extract on Sperm Quality and Testosterone Level in Mice Induced with *Staphylococcus aureus*

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ABSTRACT:

There are a variety of causes for male infertility. Among them is microbial infection. Lemongrass (C. nardus) contains natural antioxidants and flavonoids that have the ability to preserve the male reproductive system. This study examined the effects of different *C. nardus* extract dosages on mouse sperm quality. The male Balb/C mice were separated into five groups of equal size. The negative control group was injected with 0.01 ml of Na-CMC 0.5% over the course of 28 days, the positive control group was injected with 0.2 ml of *Staphylococcus aureus* (*S. aureus*) on days 10, 17, and 24, and the treatment groups were injected with 0.2 ml of *S. aureus* on days 10, 17, and 24, followed by 0.01 ml of C. nardus. After the experiment, mice were slaughtered, and sperm suspension was obtained from the cauda epididymal to measure morphology, concentration, and motility. The testis was fixed in a fixative solution to analyze the spermatogenic cells, and intracardiac blood was drawn to determine the level of testosterone. Significant differences (p 0.05) were seen in the morphology, concentration, and motility of spermatozoa when *C. nardus* extract was administered. Given the low and middle concentrations of *C. nardus* extract, it can repair sperm damage caused by S. aureus infection.

KEYWORDS: male infertility, *C. nardus* extract, *S. aureus*, sperm quality, testosterone level

INTRODUCTION

Infertility is becoming more common in Indonesia every year. Infertility affects not only women but also men. Men are responsible for 36% of fertility success1,2. Male infertility can be caused by a variety of factors, including bacteria pathogens such as Chlamydia sp., Escherichia coli, Mycoplasma hominis, and Staphylococcus aureus. This bacteria has been shown to reduce sperm motility[3,4,5]. Staphylococcus aureus (S. aureus) can cause infections in a variety of ecological niches within the host6. It colonizes the nares, axillae, vagina, pharynx, and damaged skin surfaces in humans, causing a variety of suppurative (pus-forming) infections and toxinoses7,8. Many studies have revealed that S. aureus can cause male infertility[9,10]. One study revealed that incubating *S. aureus* in sperm reduced testis weight, seminiferous tubulous diameter, epididymal sperm count, and serum testosterone level[11,12]. Another study found that S. aureus alters the composition of sperm proteins and promotes early cell death by secreting lipopolysaccharides (LPS). It resulted in an increase in dead cells and a decrease in motile sperm cells[3,13]. In this case, the use of antioxidant-containing plants is thought to reduce the toxic effect of various toxicants, including bacterial infection[14,15]. The concentration of spermatozoa in one ejaculation depends on the process of formation of spermatogenesis in seminiferous tubules16. When spermatogenesis proceeds normally it will be generated a normal spermatozoa count, otherwise during the process spermatogenesis occurs interference, then the development of spermatogonia cells will affect the number of spermatozoa formed. It depends on the magnitude of the disturbance that occurred during the process of spermatogenesis[16,17]. Lemongrass is a natural antioxidant with numerous benefits that are commonly used in herbal medicine[18]. Cymbopogon nardus, one type of lemongrass studied in this study, is known for its antioxidant activity and effectiveness to attack bacteria by lysis the cells via

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its potent flavonoid compound19,20. As a result, this study looked into the potential protective effects of C. nardus extract on the male reproductive system after infection with S. aureus. The parameters measured were epididymal and testicular
weight, the morphology of sperm, concentration of sperm, motility of sperm, spermatogenic cells, the diameter of seminiferous tubules, and testosterone level.

MATERIALS AND METHODS

Ethical approval

With the number 120-KEP-UB-2021, the Ethical Review Committee of the Research and Community Service Department at Universitas Brawijaya, East Java, Indonesia, approved all procedures in this study, including the use of mice as animal models.

Plant-based substances

C. nardus was collected in September 2021 from Materia Medica, Batu, East Java. It was acknowledged and authenticated by Universitas Muhammadiyah Lamongan's Biological Laboratory, Biology Department.

Na-CMC 0.5% preparation and suspension

A total of 500 milligrams of *Na-Carboxymethyl cellulose* (Na-CMC) were weighed and dissolved in warm distilled water. Using a 100 ml measuring flask, all of the remaining distilled water was added after dissolving to obtain a volume of 100 ml Na-CMC solution.

C. nardus ethanolic extract preparation and suspension

C. nardus stalks were cut into little pieces and dried in the sun for about five days. The 250 g of dried stalks were ground in an electric grinder before being macerated with ethanol at room temperature for three days. Then, the extract was filtered and concentrated in a water bath at 70^0 degrees Celsius. To create the suspension, the extract was homogeneously mashed with 0.5% Na-CMC. The respective concentrations were 0.5%, 1%, and 2%.

Microorganism

The strain of *S. aureus* employed in this investigation was previously obtained from the cervix of an infertile woman in our laboratory.

Substances and chemicals

Ethanol, methanol, eosin, nigrosine, chloroform (purchased from Merck, Darmstadt, Germany), distilled water, formalin 10% neutral buffer, paraffin, hematoxyline eosin, testosterone kit, xylol, alcohol 70%, alcohol 96%, and NaCl 0.9%.

Animals

Thirty mature male Balb/C mice were donated from the Veterinary and Pharma Center in Surabaya, East Java (eight weeks old, weighing 25–30 grams). They were fed and watered ad libitum in standard laboratory conditions (temperature room at 28–30 °C, and 12-h–12-h light-dark cycle).

Experimental design

After a week of acclimatization, the animals were put into five equal groups (n = 6). The negative control groups were given 0.1 ml of Na-CMC 0.5% subcutaneously every day for 28 days. The positive control groups were given 0.2 ml of S. aureus (0.5 Mc Farland) three times on days 10, 17, and 24. As positive controls, 0.2 ml S. aureus (0.5 McFarland) was given three times to the treatment groups through the intraperitoneal route. Then, different doses of 0.1 ml were given subcutaneously to each treatment group in a steady stream. The first group was given 25 mg/kg of body weight, the second group was given 50 mg/kg of body weight, and the third group was given 100 mg/kg of body weight. All three treatments were given over the duration of 28 days. At the end of the process, chloroform was used to kill all of the mice. Then, the cauda epididymal was taken and used to make a sperm suspension for measuring sperm.

Sperm analysis

This study initially measured epididymal and testicular weight from each sample, then measured the percentage of motility, morphology, and the concentration of spermatozoa in a suspension. 10µL was evaluated under a light microscope in a hemocytometer chamber for sperm motility. One hundred sperm cells were counted in each animal, and the percentage of motility was classified as motil (%) and immotile (%). The motile sperms have moved straight. An hour after sperm diffusion in the solution, 10µL of the sperm suspension was transferred to each hemocytometer counting chamber for 5 minutes to evaluate sperm concentration. Using a 40-magnification light microscope, sperm heads were counted and quantified as million/ml of suspension. Eosin-nigrosine staining determined sperm morphology. To make smears, spermatozoa suspension is dropped on the object's glass and dried in the air. Smears were fixed with methanol, colored with 1% Y eosin solution and nigrosine, and dried. Distilled water cleaned and dried the preparations. 100 mouse spermatozoa were examined using a 400-magnification light microscope. Calculating the normal/abnormal spermatozoa percentage was the final stage.

Levels of Testosterone

Serum was taken from intracardiac blood. An enzyme-linked immunoassay kit (Bioassay Technology Lab® 96 kit testosterone, China) evaluated serum testosterone. The level of testosterone measured as nmol/mL

Examining Spermatogenic Cells Histologically

After fixing the testicular collection in neutral buffer formalin 10%, paraffin, and hematoxyline eosin were used to make histological preparations with a 5µm thickness. The diameter of seminiferous tubules and the number of spermatogenic cells (spermatogonia, spermatid, and spermatocytes) were examined in mouse

testicular preparations under 400x magnification.

Data analysis

All data were represented as means standard error of the mean (SEM), and statistical analysis was conducted using one-way analysis of variance (one-way ANOVA) and post hoc multiple comparison test to determine differences between pairs of means.

RESULT

The results showed that giving S. aureus to mice for 28 days led to a significant (p0.05) decrease in the quantity of sperm and the percentage of spermatozoa that could move (immotile). In mice that were infected by *S. aureus*, the percentage of spermatozoa that had an aberrant morphology was statistically (p 0.05) higher than it was in the group that served as a negative control (K1). When compared to animals infected with *S. aureus*, testosterone levels and sperm parameters such as motility and concentration increased significantly (p 0.05) in mice that were given *C. nardus* extract (Table 1). The diameter of the seminiferous tubule, the quantity of spermatogonia cells, spermatocytes, and spermatid cells in the positive control that was infected by *S. aureus* all had lower values than the normal negative control and the group that was treated with *C. nardus* extract. This was another finding (Table 2).

According to the data in table 1, spermatozoa motility at a dose of 100 mg/kg body weight of *C. nardus* extract (P3) was not significantly different from the positive control group (K2). This means that at high doses, the ability to restore the quality of spermatozoa damaged by lead acetate exposure was not optimal. Furthermore, at a dose of 50 mg/kg body weight, the *C. nardus* extract demonstrated the highest average in each observational parameter. It is resonable to assume tat the dose was optimal for improving spermatozoa quality. A description of sperm morphological observation was shown in Figure 1. The differences in the histological seminiferous tubule in each group were shown in Figure 2.

Table 1. The effect of C. nardus extract on sperm motility, morphology, concentration, and testosterone levels in *S. aureus*-infected mice.

Parameters	K1	K2	P1	P2	P3
Motility (%)	80,60 ± 2,51^{a}	58,20 ± 2,28^{b}	70,40 ± 2,70^{ab}	76,20 ± 4,02^{a}	61,00 ± 1,58^{b}
Normal morphology (%)	83,60 ± 2,30^{a}	41,60 ± 2,07^{bc}	67,20 ± 1,92^{ab}	70,40 ± 3,36^{ab}	51,00 ± 2,92^{b}
Concentration (x10^{6}/ mL)	83,00 ± 2,55^{a}	50,00 ± 2,24^{ab}	72,20 ± 1,48^{b}	77,20 ± 1,92^{bc}	65,80 ± 2,78^{c}
Testosteron level (nmol/L)	13.68 ± 0.65^{a}	8.60 ± 0.40	10.90 ± 0.57	12.89 ± 0.85^{a}	9.69 ± 0.42
Testicular weight (mg)	306 ± 20,73^{a}	216 ± 20,73^{ab}	258 ± 23,76^{b}	292 ± 23,88^{a}	245 ± 7,07^{ab,b}
Epididymal weight (mg)	10,84 ± 0,39^{a}	7,68 ± 0,40	10,02 ± 0,58^{ab}	10,36 ± 0,63^{a,ab}	8,46 ± 0,34

Data are presented as mean ± SD. Differences in superscripts in the same rows show significant differences (p<0,05). K1 (negative control): Na-CMC 0,5%; K2 (positive control): S. aureus 0,5 Mc Farland; P1 (treatment group 1): S. aureus 0,5 Mc Farland + C. nardus 25 mg/kg BW; P2 (treatment group 2): S. aureus 0,5 Mc Farland + C. nardus 50 mg/kg BW; P3 (treatment group 3): S. aureus 0,5 Mc Farland + C. nardus 100 mg/kg BW.

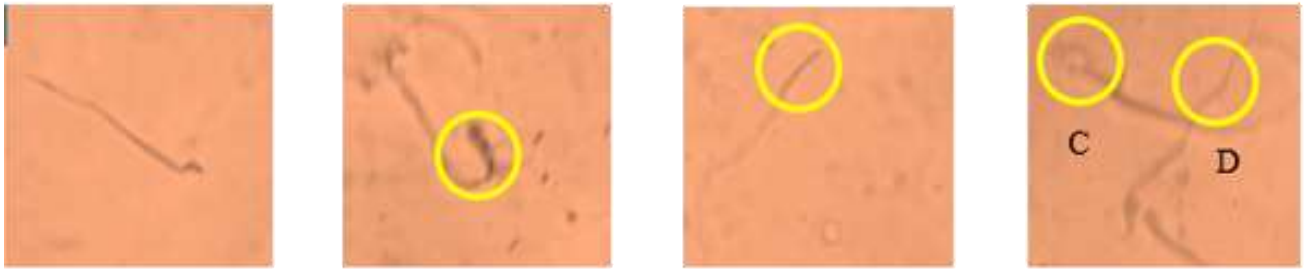

Fig.1. Sperm cell morphology in adult mice had normal (N) and abnormal (A: twisted midpiece or tail; B: head malformation (like a needle); C: larger head; D: broken tail).

Table 2. Effect of *C. nardus* extract on spermatogenic cells and diameter of the seminiferous tubule in mice infected by *S. aureus*

Parameters	K1	K2	P1	P2	P3
DST	216,52 ± 16.65[a]	105.60 ± 13.91[b]	141.41 ± 13.19	186.03 ± 29.70[a]	115.79 ± 13.85[b]
Spermatogonia cells	82,80 ± 4,38[a]	60,00 ± 4,00	75,20 ± 4,60[ab]	80,00 ± 3,16[a,ab]	67,20 ± 4,15
Spermatocytes cells	99,60 ± 5,73[a]	65,20 ± 7,95	87,80 ± 6,72[ab]	98,00 ± 6,33[a]	81,20 ± 3,35[ab]
Spermatid cells	284,0 ± 42,9[a]	179,2 ± 18,6	255,2 ± 39,5[a,ab]	268,0 ± 24,5[a,ab]	204,8 ± 15,1

Data are presented as mean ± SD

Differences in superscripts in the same rows show significant differences ($p<0.05$). K1 (negative control): Na-CMC 0,5%; K2 (positive control): *S. aureus* 0,5 Mc Farland; P1 (treatment group 1): *S. aureus* 0,5 Mc Farland + *C. nardus* 25 mg/kg BW; P2 (treatment group 2): *S. aureus* 0,5 Mc Farland + *C. nardus* 50 mg/kg BW; P3 (treatment group 3): *S. aureus* 0,5 Mc Farland + *C. nardus* 100 mg/kg BW. DST = Diameter of Seminiferous Tubules.

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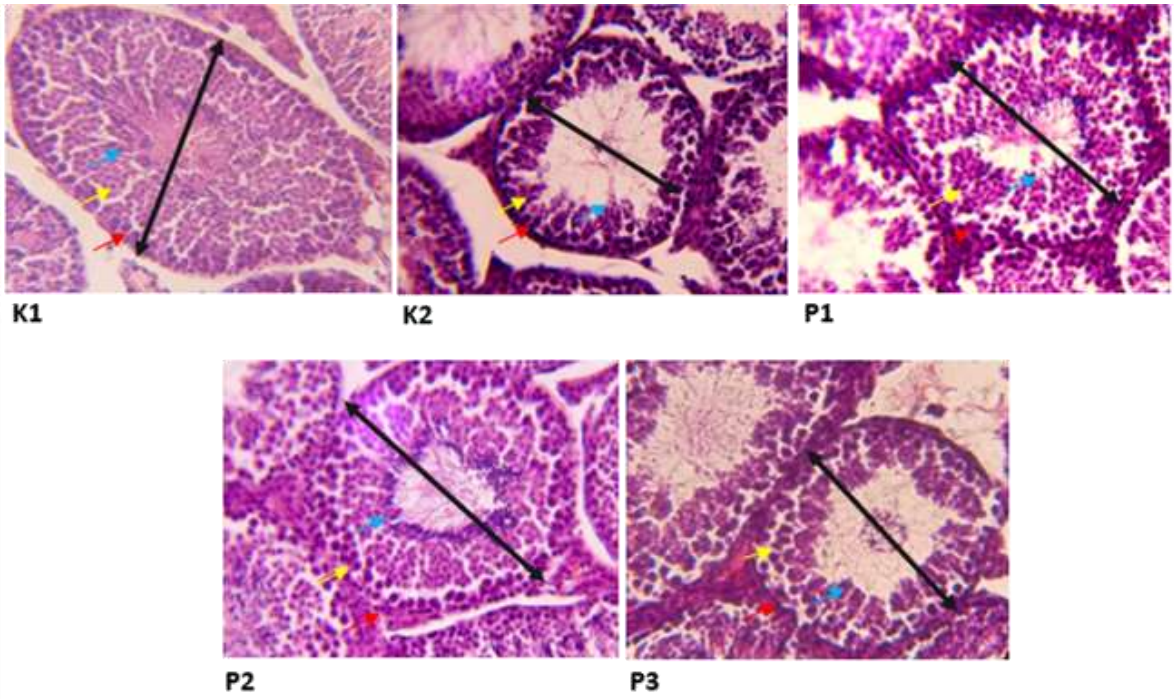

Fig. 2: Testicular seminiferous tubules in different groups. Red arrows indicate spermatogonia cells, yellow spermatocytes, blue spermatids, and black seminiferous tubule diameter. (Hematoxylin Eosin: 400x).

DISCUSSION

In ethnomedicines, the use of medicinal plants, commonly known as medicinal herbs, has been suggested for the treatment of a wide variety of disorders at various points in history and continued to be practiced today. Throughout the history of humanity, people have relied on the therapeutic properties of various plants to heal a variety of illnesses[21,22]. New scientific approaches have been integrated into conventional medical practice to facilitate the standardization of phytotherapy, which refers to the use of numerous plant-based remedies in combination with one another[23,14].

According to this study, The decreased quality of spermatozoa that has been caused by bacterial infection was partially restored by the use of an extract of *C. nardus*. There was a statistically significant difference ($p<0.05$) between the group that was treated with *C. nardus* extract and the group that served as the positive control and was treated with *S. aureus*. To acquire the optimal results in terms of epididymal and testicular weight, sperm motility, normal morphology of sperm, testosterone level, sperm concentration, and the number of spermatogenic cells, a dose of 25 mg/kg of body weight was shown to be the most effective. The increased dose that was administered did not result in an improvement in the quality of the spermatozoa. Even at a dose of 100 mg/kg of body weight, the quality of sperm that had been infected with *S. aureus* was not improved.

S. aureus is a pathogen bacteria that are typically found in the female genital tract[2]. This bacteria is known to have a toxic effect on the epididymal duct in addition to disrupting the process of spermatogenesis and also could well interact with the CD14 on the surface of the sperm,

primarily in the head and tail region[24]. This interaction will result in increased levels of sperm membrane lipid peroxidation, production of reactive oxygen species, and caspase-mediated apoptosis in the subsequent stages[6,10,25]. A reduction in the motility of spermatozoa and an increase in lipid peroxidation are both indicators that cell damage has been caused *by S. aureus*[26,16,27]

The process of male reproduction is a complicated one that requires a great deal of energy. The hypothalamus and the pituitary gland use a variety of feedback regulation mechanisms to control the function of the testis, which serves as the primary organ of the male germline[1,28]. GnRH, FSH, and LH are the primary hormones responsible for controlling regulatory mechanisms via their actions on the hypothalamus-pituitary-testis axis[29]. Through neurons that secrete GnRH, the hypothalamus can monitor the condition of the reproductive system. Through the anterior pituitary, GnRH stimulates the release of LH as well as FSH. LH stimulates the production of testosterone by acting on testicular interstitial cells and increasing their permeability. FSH is the hormone that is responsible for the stimulation of the seminiferous tubules, which is necessary to maintain sperm production[30].

A decrease in the amount of testosterone in the body can lead to reproductive disorders. Testosterone is a hormone that is primarily found in men[31]. The precise and coordinated secretion of GnRH, LH, FSH, and testosterone is what regulates male reproductive function. Testosterone also plays a role in this regulation. The production of spermatozoa as well as the synthesis and release of testosterone are both the responsibility of the testes[32]. A lower testosterone level may be associated with a more complex condition, such as erectile dysfunction[33,34]. Phosphodiesterase 5 (PDE-5) inhibitors, such as Sildenafil, Tadalafil, and others, are typically prescribed to patients who have been diagnosed with erectile dysfunction. According to several studies, the effectiveness of PDE-5 inhibitors is improved when there is an adequate quantity of testosterone in the body[35]. Erectile dysfunction may be caused by several causes, but the one that is caused by a lack of testosterone is likely treatable by using medicines that raise testosterone levels[33]. Other causes of erectile dysfunction may not be so easily remedied. In the current study, after administering a treatment consisting of *C. nardus* extract for a period of 14 days, significant increases in testosterone levels were observed in the serum.

One of the herbal medications that have been shown in a recent study to have the potential impact of protecting organs against free radicals is called *C. nardus*[36,19]. Antioxidants, flavonoids, phenolics, and terpenoids are the types of phytochemical substances that can be found in *C. nardus*[37,38]. Antioxidants shield lipid membranes against damage caused by superoxide, ammonium, and peroxide radicals, which are each capable of causing cellular degeneration[39,40]. Antioxidants also participate in the electron transport process that occurs during the final step of energy production in mitochondria[41]. To improve sperm motility, mitochondria must produce ATP or other forms of energy before fertilization may proceed[42].

Fertilization is dependent on several factors, one of which is motility. If motility were to suffer any kind of impairment, this would have a negative influence on fertilization capacity[43]. During their journey through the epididymis, sperm develop the ability to move about, and the epididymis is responsible for the initialization of this ability by both providing a distinct microenvironment and also secreting proteins that are essential for the initialization of sperm motility[21,44].

In the previous research, the protective effect of lemongrass in male reproduction after being triggered by hydrogen peroxide was described. And the results demonstrated that an increase in testosterone level, sperm characteristics, testicular and epididymal weight, as well as a reduction in serum and tissue homogenate *malondialdehyde* (MDA) and testicular histopathological changes, can be brought about by administering 100 mg of lemongrass extract per kg of body weight[45]. In correlation with the previous research, we found that *C. nardus* extract could improve sperm quality in mice after being induced by *S. aureus*. The potential dose of *C. nardus* that could improve sperm quality significantly was 25 mg/kg of body weight, and this was found to be the optimal amount for this improvement.

CONCLUSION

The *C. nardus* extract protects spermatozoa cells by repairing the sperm quality decrease caused by *S. aureus* infection. A low to a middle dose of *C. nardus* extract provides more protection than a high dose. A high dose of *C. nardus* extract is ineffective at improving sperm motility and concentration.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this research.

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LEMBAR
HASIL PENILAIAN SEJAWAT SEBIDANG ATAU *PEER REVIEW*
KARYA ILMIAH : JURNAL ILMIAH

Judul Jurnal Ilmiah (Artikel)	:	*"Examining the Effects of Lemongrass (Cymbopogon nardus) Extract on Sperm Quality and Testosterone Level in Mice Induced with Staphylococcus aureus"*
Jumlah Penulis	:	6 orang
Status Pengusul	:	~~Penulis Utama~~/Penulis ke-2 /Penulis korespondensi
Identitas Jurnal Ilmiah	:	i. Nama Jurnal : RJPT: Research Journal of Pharmaceutical and Technology j. Nomor ISSN : Online ISSN: 0974-360X Print ISSN: 0974-3618 k. Nomor/Volume : Volume 16 / Nomor 10 l. Edisi (bulan/tahun) : Oktober, 2023 m. Penerbit : A and V Publication n. DOI artikel/ tautan eksternal : https://drive.google.com/file/d/1-BzHkaBtndlrP5Q1CunHCj4QKV7i7WfA/view?usp=sharing o. Alamat Web Jurnal : https://www.rjptonline.org/AboutJournal.aspx p. Jumlah halaman : 7 Halaman
	:	☑ Jurnal Internasional Terindeks basis data internasional bereputasi dan berimpact faktor ☐ Jurnal Nasional Terakreditasi/ Peringkat 1 dan 2 SINTA ☐ Jurnal Nasional DOAJ/CABI/COPERNICUS/Peringkat 3 dan 4 SINTA ☐ Jurnal Nasional Peringkat 5 dan 6 SINTA

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Komponen Yang Dinilai	Nilai Maksimal Jurnal Ilmiah				Nilai Akhir Yang Diperoleh (NA)
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e. Kelengkapan unsur isi artikel (10%)	4	2,5	2	1,5	**3,6**
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Total = (100%)	40	25	20	15	**39,3**
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Penulis pertama = 0,4; Penulis koresponding = 0,4
Anggota = 0,4/ 0,2 dibagi banyak anggota

Lamongan, 10 Januari 2024
Reviewer 1,

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Jabatan Akademik Terakhir: Guru Besar
Bidang Ilmu : Biologi/ Fisiologi Hewan

LEMBAR
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h. Kelengkapan unsur dan kualitas terbitan/jurnal (30%)	12	7,5	6	4,5	**11,7**
Total = (100%)	40	25	20	15	**39,4**
Nilai Pengusul (NA x BP)	**39,4 x 0,4=**	**15,76**			

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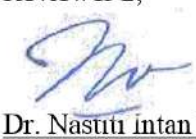

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Examining the Effects of Lemongrass (Cymbopogon nardus) Extract on Sperm

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RESEARCH ARTICLE

Examining the Effects of Lemongrass (*Cymbopogon nardus*) Extract on Sperm Quality and Testosterone Level in Mice Induced with *Staphylococcus aureus*

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ABSTRACT:

There are a variety of causes for male infertility. Among them is microbial infection. Lemongrass (C. nardus) contains natural antioxidants and flavonoids that have the ability to preserve the male reproductive system. This study examined the effects of different *C. nardus* extract dosages on mouse sperm quality. The male Balb/C mice were separated into five groups of equal size. The negative control group was injected with 0.01 ml of Na-CMC 0.5% over the course of 28 days, the positive control group was injected with 0.2 ml of *Staphylococcus aureus* (*S. aureus*) on days 10, 17, and 24, and the treatment groups were injected with 0.2 ml of *S. aureus* on days 10, 17, and 24, followed by 0.01 ml of C. nardus. After the experiment, mice were slaughtered, and sperm suspension was obtained from the cauda epididymal to measure morphology, concentration, and motility. The testis was fixed in a fixative solution to analyze the spermatogenic cells, and intracardiac blood was drawn to determine the level of testosterone. Significant differences (p 0.05) were seen in the morphology, concentration, and motility of spermatozoa when *C. nardus* extract was administered. Given the low and middle concentrations of *C. nardus* extract, it can repair sperm damage caused by S. aureus infection.

KEYWORDS: male infertility, *C. nardus* extract, *S. aureus*, sperm quality, testosterone level

INTRODUCTION

Infertility is becoming more common in Indonesia every year. Infertility affects not only women but also men. Men are responsible for 36% of fertility success1,2. Male infertility can be caused by a variety of factors, including bacteria pathogens such as Chlamydia sp., Escherichia coli, Mycoplasma hominis, and Staphylococcus aureus. This bacteria has been shown to reduce sperm motility[3,4,5]. Staphylococcus aureus (S. aureus) can cause infections in a variety of ecological niches within the host6. It colonizes the nares, axillae, vagina, pharynx, and damaged skin surfaces in humans, causing a variety of suppurative (pus-forming) infections and toxinoses7,8. Many studies have revealed that S. aureus can cause male infertility[9,10]. One study revealed that incubating *S. aureus* in sperm reduced testis weight, seminiferous tubulous diameter, epididymal sperm count, and serum testosterone level[11,12]. Another study found that S. aureus alters the composition of sperm proteins and promotes early cell death by secreting lipopolysaccharides (LPS). It resulted in an increase in dead cells and a decrease in motile sperm cells[3,13]. In this case, the use of antioxidant-containing plants is thought to reduce the toxic effect of various toxicants, including bacterial infection[14,15]. The concentration of spermatozoa in one ejaculation depends on the process of formation of spermatogenesis in seminiferous tubules16. When spermatogenesis proceeds normally it will be generated a normal spermatozoa count, otherwise during the process spermatogenesis occurs interference, then the development of spermatogonia cells will affect the number of spermatozoa formed. It depends on the magnitude of the disturbance that occurred during the process of spermatogenesis[16,17]. Lemongrass is a natural antioxidant with numerous benefits that are commonly used in herbal medicine[18]. Cymbopogon nardus, one type of lemongrass studied in this study, is known for its antioxidant activity and effectiveness to attack bacteria by lysis the cells via

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its potent flavonoid compound19,20. As a result, this study looked into the potential protective effects of C. nardus extract on the male reproductive system after infection with S. aureus. The parameters measured were epididymal and testicular
weight, the morphology of sperm, concentration of sperm, motility of sperm, spermatogenic cells, the diameter of seminiferous tubules, and testosterone level.

MATERIALS AND METHODS

Ethical approval

With the number 120-KEP-UB-2021, the Ethical Review Committee of the Research and Community Service Department at Universitas Brawijaya, East Java, Indonesia, approved all procedures in this study, including the use of mice as animal models.

Plant-based substances

C. nardus was collected in September 2021 from Materia Medica, Batu, East Java. It was acknowledged and authenticated by Universitas Muhammadiyah Lamongan's Biological Laboratory, Biology Department.

Na-CMC 0.5% preparation and suspension

A total of 500 milligrams of *Na-Carboxymethyl cellulose* (Na-CMC) were weighed and dissolved in warm distilled water. Using a 100 ml measuring flask, all of the remaining distilled water was added after dissolving to obtain a volume of 100 ml Na-CMC solution.

C. nardus ethanolic extract preparation and suspension

C. nardus stalks were cut into little pieces and dried in the sun for about five days. The 250 g of dried stalks were ground in an electric grinder before being macerated with ethanol at room temperature for three days. Then, the extract was filtered and concentrated in a water bath at 70^0 degrees Celsius. To create the suspension, the extract was homogeneously mashed with 0.5% Na-CMC. The respective concentrations were 0.5%, 1%, and 2%.

Microorganism

The strain of *S. aureus* employed in this investigation was previously obtained from the cervix of an infertile woman in our laboratory.

Substances and chemicals

Ethanol, methanol, eosin, nigrosine, chloroform (purchased from Merck, Darmstadt, Germany), distilled water, formalin 10% neutral buffer, paraffin, hematoxyline eosin, testosterone kit, xylol, alcohol 70%, alcohol 96%, and NaCl 0.9%.

Animals

Thirty mature male Balb/C mice were donated from the Veterinary and Pharma Center in Surabaya, East Java (eight weeks old, weighing 25–30 grams). They were fed and watered ad libitum in standard laboratory conditions (temperature room at 28–30 °C, and 12-h–12-h light-dark cycle).

Experimental design

After a week of acclimatization, the animals were put into five equal groups (n = 6). The negative control groups were given 0.1 ml of Na-CMC 0.5% subcutaneously every day for 28 days. The positive control groups were given 0.2 ml of S. aureus (0.5 Mc Farland) three times on days 10, 17, and 24. As positive controls, 0.2 ml S. aureus (0.5 McFarland) was given three times to the treatment groups through the intraperitoneal route. Then, different doses of 0.1 ml were given subcutaneously to each treatment group in a steady stream. The first group was given 25 mg/kg of body weight, the second group was given 50 mg/kg of body weight, and the third group was given 100 mg/kg of body weight. All three treatments were given over the duration of 28 days. At the end of the process, chloroform was used to kill all of the mice. Then, the cauda epididymal was taken and used to make a sperm suspension for measuring sperm.

Sperm analysis

This study initially measured epididymal and testicular weight from each sample, then measured the percentage of motility, morphology, and the concentration of spermatozoa in a suspension. 10µL was evaluated under a light microscope in a hemocytometer chamber for sperm motility. One hundred sperm cells were counted in each animal, and the percentage of motility was classified as motil (%) and immotile (%). The motile sperms have moved straight. An hour after sperm diffusion in the solution, 10µL of the sperm suspension was transferred to each hemocytometer counting chamber for 5 minutes to evaluate sperm concentration. Using a 40-magnification light microscope, sperm heads were counted and quantified as million/ml of suspension. Eosin-nigrosine staining determined sperm morphology. To make smears, spermatozoa suspension is dropped on the object's glass and dried in the air. Smears were fixed with methanol, colored with 1% Y eosin solution and nigrosine, and dried. Distilled water cleaned and dried the preparations. 100 mouse spermatozoa were examined using a 400-magnification light microscope. Calculating the normal/abnormal spermatozoa percentage was the final stage.

Levels of Testosterone

Serum was taken from intracardiac blood. An enzyme-linked immunoassay kit (Bioassay Technology Lab® 96 kit testosterone, China) evaluated serum testosterone. The level of testosterone measured as nmol/mL

Examining Spermatogenic Cells Histologically

After fixing the testicular collection in neutral buffer formalin 10%, paraffin, and hematoxyline eosin were used to make histological preparations with a 5µm thickness. The diameter of seminiferous tubules and the number of spermatogenic cells (spermatogonia, spermatid, and spermatocytes) were examined in mouse

testicular preparations under 400x magnification.

Data analysis

All data were represented as means standard error of the mean (SEM), and statistical analysis was conducted using one-way analysis of variance (one-way ANOVA) and post hoc multiple comparison test to determine differences between pairs of means.

RESULT

The results showed that giving S. aureus to mice for 28 days led to a significant (p0.05) decrease in the quantity of sperm and the percentage of spermatozoa that could move (immotile). In mice that were infected by *S. aureus*, the percentage of spermatozoa that had an aberrant morphology was statistically (p 0.05) higher than it was in the group that served as a negative control (K1). When compared to animals infected with *S. aureus*, testosterone levels and sperm parameters such as motility and concentration increased significantly (p 0.05) in mice that were given *C. nardus* extract (Table 1). The diameter of the seminiferous tubule, the quantity of spermatogonia cells, spermatocytes, and spermatid cells in the positive control that was infected by *S. aureus* all had lower values than the normal negative control and the group that was treated with *C. nardus* extract. This was another finding (Table 2).

According to the data in table 1, spermatozoa motility at a dose of 100 mg/kg body weight of *C. nardus* extract (P3) was not significantly different from the positive control group (K2). This means that at high doses, the ability to restore the quality of spermatozoa damaged by lead acetate exposure was not optimal. Furthermore, at a dose of 50 mg/kg body weight, the *C. nardus* extract demonstrated the highest average in each observational parameter. It is resonable to assume tat the dose was optimal for improving spermatozoa quality. A description of sperm morphological observation was shown in Figure 1. The differences in the histological seminiferous tubule in each group were shown in Figure 2.

Table 1. The effect of C. nardus extract on sperm motility, morphology, concentration, and testosterone levels in *S. aureus*-infected mice.

Parameters	K1	K2	P1	P2	P3
Motility (%)	80,60 ± 2,51^{a}	58,20 ± 2,28^{b}	70,40 ± 2,70^{ab}	76,20 ± 4,02^{a}	61,00 ± 1,58^{b}
Normal morphology (%)	83,60 ± 2,30^{a}	41,60 ± 2,07^{bc}	67,20 ± 1,92^{ab}	70,40 ± 3,36^{ab}	51,00 ± 2,92^{b}
Concentration (x10^{6}/ mL)	83,00 ± 2,55^{a}	50,00 ± 2,24^{ab}	72,20 ± 1,48^{b}	77,20 ± 1,92^{bc}	65,80 ± 2,78^{c}
Testosteron level (nmol/L)	13.68 ± 0.65^{a}	8.60 ± 0.40	10.90 ± 0.57	12.89 ± 0.85^{a}	9.69 ± 0.42
Testicular weight (mg)	306 ± 20,73^{a}	216 ± 20,73^{ab}	258 ± 23,76^{b}	292 ± 23,88^{a}	245 ± 7,07^{ab,b}
Epididymal weight (mg)	10,84 ± 0,39^{a}	7,68 ± 0,40	10,02 ± 0,58^{ab}	10,36 ± 0,63^{a,ab}	8,46 ± 0,34

Data are presented as mean ± SD. Differences in superscripts in the same rows show significant differences (p<0,05). K1 (negative control): Na-CMC 0,5%; K2 (positive control): S. aureus 0,5 Mc Farland; P1 (treatment group 1): S. aureus 0,5 Mc Farland + C. nardus 25 mg/kg BW; P2 (treatment group 2): S. aureus 0,5 Mc Farland + C. nardus 50 mg/kg BW; P3 (treatment group 3): S. aureus 0,5 Mc Farland + C. nardus 100 mg/kg BW.

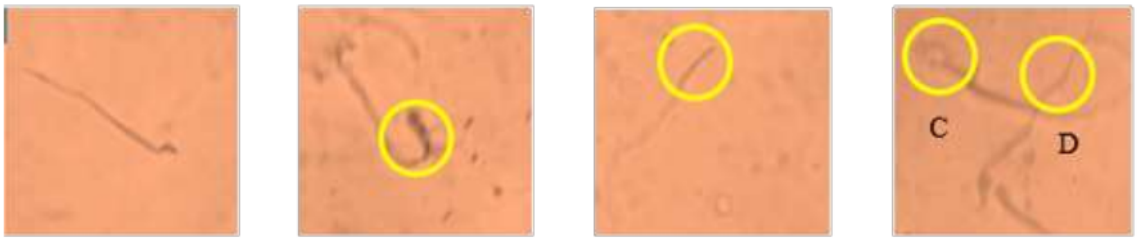

Fig.1. Sperm cell morphology in adult mice had normal (N) and abnormal (A: twisted midpiece or tail; B: head malformation (like a needle); C: larger head; D: broken tail).

Table 2. Effect of *C. nardus* extract on spermatogenic cells and diameter of the seminiferous tubule in mice infected by *S. aureus*

Parameters	K1	K2	P1	P2	P3
DST	216,52 ± 16.65[a]	105.60 ± 13.91[b]	141.41 ± 13.19	186.03 ± 29.70[a]	115.79 ± 13.85[b]
Spermatogonia cells	82,80 ± 4,38[a]	60,00 ± 4,00	75,20 ± 4,60[ab]	80,00 ± 3,16[a,ab]	67,20 ± 4,15
Spermatocytes cells	99,60 ± 5,73[a]	65,20 ± 7,95	87,80 ± 6,72[ab]	98,00 ± 6,33[a]	81,20 ± 3,35[ab]
Spermatid cells	284,0 ± 42,9[a]	179,2 ± 18,6	255,2 ± 39,5[a,ab]	268,0 ± 24,5[a,ab]	204,8 ± 15,1

Data are presented as mean ± SD
Differences in superscripts in the same rows show significant differences ($p<0.05$). K1 (negative control): Na-CMC 0,5%; K2 (positive control): *S. aureus* 0,5 Mc Farland; P1 (treatment group 1): *S. aureus* 0,5 Mc Farland + *C. nardus* 25 mg/kg BW; P2 (treatment group 2): *S. aureus* 0,5 Mc Farland + *C. nardus* 50 mg/kg BW; P3 (treatment group 3): *S. aureus* 0,5 Mc Farland + *C. nardus* 100 mg/kg BW. DST = Diameter of Seminiferous Tubules.

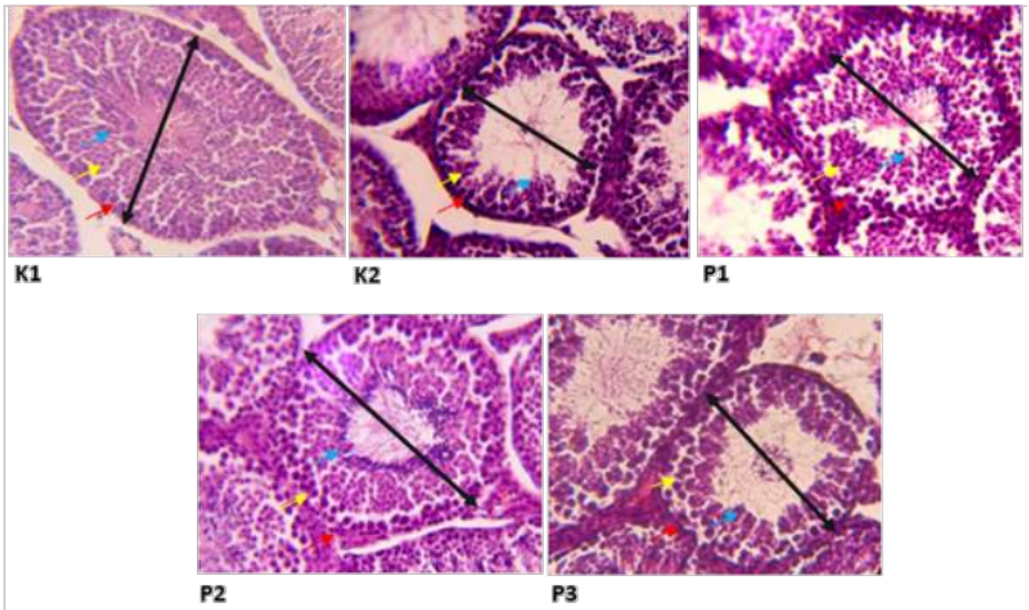

Fig. 2: Testicular seminiferous tubules in different groups. Red arrows indicate spermatogonia cells, yellow spermatocytes, blue spermatids, and black seminiferous tubule diameter. (Hematoxylin Eosin: 400x).

DISCUSSION

In ethnomedicines, the use of medicinal plants, commonly known as medicinal herbs, has been suggested for the treatment of a wide variety of disorders at various points in history and continued to be practiced today. Throughout the history of humanity, people have relied on the therapeutic properties of various plants to heal a variety of illnesses[21,22]. New scientific approaches have been integrated into conventional medical practice to facilitate the standardization of phytotherapy, which refers to the use of numerous plant-based remedies in combination with one another[23,14].

According to this study, The decreased quality of spermatozoa that has been caused by bacterial infection was partially restored by the use of an extract of *C. nardus*. There was a statistically significant difference ($p<0.05$) between the group that was treated with *C. nardus* extract and the group that served as the positive control and was treated with *S. aureus*. To acquire the optimal results in terms of epididymal and testicular weight, sperm motility, normal morphology of sperm, testosterone level, sperm concentration, and the number of spermatogenic cells, a dose of 25 mg/kg of body weight was shown to be the most effective. The increased dose that was administered did not result in an improvement in the quality of the spermatozoa. Even at a dose of 100 mg/kg of body weight, the quality of sperm that had been infected with *S. aureus* was not improved.

S. aureus is a pathogen bacteria that are typically found in the female genital tract[2]. This bacteria is known to have a toxic effect on the epididymal duct in addition to disrupting the process of spermatogenesis and also could well interact with the CD14 on the surface of the sperm,

primarily in the head and tail region[24]. This interaction will result in increased levels of sperm membrane lipid peroxidation, production of reactive oxygen species, and caspase-mediated apoptosis in the subsequent stages[6,10,25]. A reduction in the motility of spermatozoa and an increase in lipid peroxidation are both indicators that cell damage has been caused *by S. aureus*[26,16,27]

The process of male reproduction is a complicated one that requires a great deal of energy. The hypothalamus and the pituitary gland use a variety of feedback regulation mechanisms to control the function of the testis, which serves as the primary organ of the male germline[1,28]. GnRH, FSH, and LH are the primary hormones responsible for controlling regulatory mechanisms via their actions on the hypothalamus-pituitary-testis axis[29]. Through neurons that secrete GnRH, the hypothalamus can monitor the condition of the reproductive system. Through the anterior pituitary, GnRH stimulates the release of LH as well as FSH. LH stimulates the production of testosterone by acting on testicular interstitial cells and increasing their permeability. FSH is the hormone that is responsible for the stimulation of the seminiferous tubules, which is necessary to maintain sperm production[30].

A decrease in the amount of testosterone in the body can lead to reproductive disorders. Testosterone is a hormone that is primarily found in men[31]. The precise and coordinated secretion of GnRH, LH, FSH, and testosterone is what regulates male reproductive function. Testosterone also plays a role in this regulation. The production of spermatozoa as well as the synthesis and release of testosterone are both the responsibility of the testes[32]. A lower testosterone level may be associated with a more complex condition, such as erectile dysfunction[33,34]. Phosphodiesterase 5 (PDE-5) inhibitors, such as Sildenafil, Tadalafil, and others, are typically prescribed to patients who have been diagnosed with erectile dysfunction. According to several studies, the effectiveness of PDE-5 inhibitors is improved when there is an adequate quantity of testosterone in the body[35]. Erectile dysfunction may be caused by several causes, but the one that is caused by a lack of testosterone is likely treatable by using medicines that raise testosterone levels[33]. Other causes of erectile dysfunction may not be so easily remedied. In the current study, after administering a treatment consisting of *C. nardus* extract for a period of 14 days, significant increases in testosterone levels were observed in the serum.

One of the herbal medications that have been shown in a recent study to have the potential impact of protecting organs against free radicals is called *C. nardus*[36,19]. Antioxidants, flavonoids, phenolics, and terpenoids are the types of phytochemical substances that can be found in *C. nardus*[37,38]. Antioxidants shield lipid membranes against damage caused by superoxide, ammonium, and peroxide radicals, which are each capable of causing cellular degeneration[39,40]. Antioxidants also participate in the electron transport process that occurs during the final step of energy production in mitochondria[41]. To improve sperm motility, mitochondria must produce ATP or other forms of energy before fertilization may proceed[42].

Fertilization is dependent on several factors, one of which is motility. If motility were to suffer any kind of impairment, this would have a negative influence on fertilization capacity[43]. During their journey through the epididymis, sperm develop the ability to move about, and the epididymis is responsible for the initialization of this ability by both providing a distinct microenvironment and also secreting proteins that are essential for the initialization of sperm motility[21,44].

In the previous research, the protective effect of lemongrass in male reproduction after being triggered by hydrogen peroxide was described. And the results demonstrated that an increase in testosterone level, sperm characteristics, testicular and epididymal weight, as well as a reduction in serum and tissue homogenate *malondialdehyde* (MDA) and testicular histopathological changes, can be brought about by administering 100 mg of lemongrass extract per kg of body weight[45]. In correlation with the previous research, we found that *C. nardus* extract could improve sperm quality in mice after being induced by *S. aureus*. The potential dose of *C. nardus* that could improve sperm quality significantly was 25 mg/kg of body weight, and this was found to be the optimal amount for this improvement.

CONCLUSION

The *C. nardus* extract protects spermatozoa cells by repairing the sperm quality decrease caused by *S. aureus* infection. A low to a middle dose of *C. nardus* extract provides more protection than a high dose. A high dose of *C. nardus* extract is ineffective at improving sperm motility and concentration.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this research.

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RESEARCH ARTICLE

Examining the Effects of Lemongrass (*Cymbopogon nardus*) Extract on Sperm Quality and Testosterone Level in Mice Induced with *Staphylococcus aureus*

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ABSTRACT:

There are a variety of causes for male infertility. Among them is microbial infection. Lemongrass (C. nardus) contains natural antioxidants and flavonoids that have the ability to preserve the male reproductive system. This study examined the effects of different *C. nardus* extract dosages on mouse sperm quality. The male Balb/C mice were separated into five groups of equal size. The negative control group was injected with 0.01 ml of Na-CMC 0.5% over the course of 28 days, the positive control group was injected with 0.2 ml of *Staphylococcus aureus* (*S. aureus*) on days 10, 17, and 24, and the treatment groups were injected with 0.2 ml of *S. aureus* on days 10, 17, and 24, followed by 0.01 ml of C. nardus. After the experiment, mice were slaughtered, and sperm suspension was obtained from the cauda epididymal to measure morphology, concentration, and motility. The testis was fixed in a fixative solution to analyze the spermatogenic cells, and intracardiac blood was drawn to determine the level of testosterone. Significant differences (p 0.05) were seen in the morphology, concentration, and motility of spermatozoa when *C. nardus* extract was administered. Given the low and middle concentrations of *C. nardus* extract, it can repair sperm damage caused by S. aureus infection.

KEYWORDS: male infertility, *C. nardus* extract, *S. aureus*, sperm quality, testosterone level

INTRODUCTION

Infertility is becoming more common in Indonesia every year. Infertility affects not only women but also men. Men are responsible for 36% of fertility success1,2. Male infertility can be caused by a variety of factors, including bacteria pathogens such as Chlamydia sp., Escherichia coli, Mycoplasma hominis, and Staphylococcus aureus. This bacteria has been shown to reduce sperm motility[3,4,5]. Staphylococcus aureus (S. aureus) can cause infections in a variety of ecological niches within the host6. It colonizes the nares, axillae, vagina, pharynx, and damaged skin surfaces in humans, causing a variety of suppurative (pus-forming) infections and toxinoses7,8. Many studies have revealed that S. aureus can cause male infertility[9,10]. One study revealed that incubating *S. aureus* in sperm reduced testis weight, seminiferous tubulous diameter, epididymal sperm count, and serum testosterone level[11,12]. Another study found that S. aureus alters the composition of sperm proteins and promotes early cell death by secreting lipopolysaccharides (LPS). It resulted in an increase in dead cells and a decrease in motile sperm cells[3,13]. In this case, the use of antioxidant-containing plants is thought to reduce the toxic effect of various toxicants, including bacterial infection[14,15]. The concentration of spermatozoa in one ejaculation depends on the process of formation of spermatogenesis in seminiferous tubules16. When spermatogenesis proceeds normally it will be generated a normal spermatozoa count, otherwise during the process spermatogenesis occurs interference, then the development of spermatogonia cells will affect the number of spermatozoa formed. It depends on the magnitude of the disturbance that occurred during the process of spermatogenesis[16,17]. Lemongrass is a natural antioxidant with numerous benefits that are commonly used in herbal medicine[18]. Cymbopogon nardus, one type of lemongrass studied in this study, is known for its antioxidant activity and effectiveness to attack bacteria by lysis the cells via

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its potent flavonoid compound19,20. As a result, this study looked into the potential protective effects of C. nardus extract on the male reproductive system after infection with S. aureus. The parameters measured were epididymal and testicular
weight, the morphology of sperm, concentration of sperm, motility of sperm, spermatogenic cells, the diameter of seminiferous tubules, and testosterone level.

MATERIALS AND METHODS

Ethical approval

With the number 120-KEP-UB-2021, the Ethical Review Committee of the Research and Community Service Department at Universitas Brawijaya, East Java, Indonesia, approved all procedures in this study, including the use of mice as animal models.

Plant-based substances

C. nardus was collected in September 2021 from Materia Medica, Batu, East Java. It was acknowledged and authenticated by Universitas Muhammadiyah Lamongan's Biological Laboratory, Biology Department.

Na-CMC 0.5% preparation and suspension

A total of 500 milligrams of *Na-Carboxymethyl cellulose* (Na-CMC) were weighed and dissolved in warm distilled water. Using a 100 ml measuring flask, all of the remaining distilled water was added after dissolving to obtain a volume of 100 ml Na-CMC solution.

C. nardus ethanolic extract preparation and suspension

C. nardus stalks were cut into little pieces and dried in the sun for about five days. The 250 g of dried stalks were ground in an electric grinder before being macerated with ethanol at room temperature for three days. Then, the extract was filtered and concentrated in a water bath at 70^0 degrees Celsius. To create the suspension, the extract was homogeneously mashed with 0.5% Na-CMC. The respective concentrations were 0.5%, 1%, and 2%.

Microorganism

The strain of *S. aureus* employed in this investigation was previously obtained from the cervix of an infertile woman in our laboratory.

Substances and chemicals

Ethanol, methanol, eosin, nigrosine, chloroform (purchased from Merck, Darmstadt, Germany), distilled water, formalin 10% neutral buffer, paraffin, hematoxyline eosin, testosterone kit, xylol, alcohol 70%, alcohol 96%, and NaCl 0.9%.

Animals

Thirty mature male Balb/C mice were donated from the Veterinary and Pharma Center in Surabaya, East Java (eight weeks old, weighing 25–30 grams). They were fed and watered ad libitum in standard laboratory conditions (temperature room at 28–30 °C, and 12-h–12-h light-dark cycle).

Experimental design

After a week of acclimatization, the animals were put into five equal groups (n = 6). The negative control groups were given 0.1 ml of Na-CMC 0.5% subcutaneously every day for 28 days. The positive control groups were given 0.2 ml of S. aureus (0.5 Mc Farland) three times on days 10, 17, and 24. As positive controls, 0.2 ml S. aureus (0.5 McFarland) was given three times to the treatment groups through the intraperitoneal route. Then, different doses of 0.1 ml were given subcutaneously to each treatment group in a steady stream. The first group was given 25 mg/kg of body weight, the second group was given 50 mg/kg of body weight, and the third group was given 100 mg/kg of body weight. All three treatments were given over the duration of 28 days. At the end of the process, chloroform was used to kill all of the mice. Then, the cauda epididymal was taken and used to make a sperm suspension for measuring sperm.

Sperm analysis

This study initially measured epididymal and testicular weight from each sample, then measured the percentage of motility, morphology, and the concentration of spermatozoa in a suspension. 10µL was evaluated under a light microscope in a hemocytometer chamber for sperm motility. One hundred sperm cells were counted in each animal, and the percentage of motility was classified as motil (%) and immotile (%). The motile sperms have moved straight. An hour after sperm diffusion in the solution, 10µL of the sperm suspension was transferred to each hemocytometer counting chamber for 5 minutes to evaluate sperm concentration. Using a 40-magnification light microscope, sperm heads were counted and quantified as million/ml of suspension. Eosin-nigrosine staining determined sperm morphology. To make smears, spermatozoa suspension is dropped on the object's glass and dried in the air. Smears were fixed with methanol, colored with 1% Y eosin solution and nigrosine, and dried. Distilled water cleaned and dried the preparations. 100 mouse spermatozoa were examined using a 400-magnification light microscope. Calculating the normal/abnormal spermatozoa percentage was the final stage.

Levels of Testosterone

Serum was taken from intracardiac blood. An enzyme-linked immunoassay kit (Bioassay Technology Lab® 96 kit testosterone, China) evaluated serum testosterone. The level of testosterone measured as nmol/mL

Examining Spermatogenic Cells Histologically

After fixing the testicular collection in neutral buffer formalin 10%, paraffin, and hematoxyline eosin were used to make histological preparations with a 5µm thickness. The diameter of seminiferous tubules and the number of spermatogenic cells (spermatogonia, spermatid, and spermatocytes) were examined in mouse

testicular preparations under 400x magnification.

Data analysis

All data were represented as means standard error of the mean (SEM), and statistical analysis was conducted using one-way analysis of variance (one-way ANOVA) and post hoc multiple comparison test to determine differences between pairs of means.

RESULT

The results showed that giving S. aureus to mice for 28 days led to a significant (p0.05) decrease in the quantity of sperm and the percentage of spermatozoa that could move (immotile). In mice that were infected by *S. aureus*, the percentage of spermatozoa that had an aberrant morphology was statistically (p 0.05) higher than it was in the group that served as a negative control (K1). When compared to animals infected with *S. aureus*, testosterone levels and sperm parameters such as motility and concentration increased significantly (p 0.05) in mice that were given *C. nardus* extract (Table 1). The diameter of the seminiferous tubule, the quantity of spermatogonia cells, spermatocytes, and spermatid cells in the positive control that was infected by *S. aureus* all had lower values than the normal negative control and the group that was treated with *C. nardus* extract. This was another finding (Table 2).

According to the data in table 1, spermatozoa motility at a dose of 100 mg/kg body weight of *C. nardus* extract (P3) was not significantly different from the positive control group (K2). This means that at high doses, the ability to restore the quality of spermatozoa damaged by lead acetate exposure was not optimal. Furthermore, at a dose of 50 mg/kg body weight, the *C. nardus* extract demonstrated the highest average in each observational parameter. It is resonable to assume tat the dose was optimal for improving spermatozoa quality. A description of sperm morphological observation was shown in Figure 1. The differences in the histological seminiferous tubule in each group were shown in Figure 2.

Table 1. The effect of C. nardus extract on sperm motility, morphology, concentration, and testosterone levels in *S. aureus*-infected mice.

Parameters	K1	K2	P1	P2	P3
Motility (%)	80,60 ± 2,51^{a}	58,20 ± 2,28^{b}	70,40 ± 2,70^{ab}	76,20 ± 4,02^{a}	61,00 ± 1,58^{b}
Normal morphology (%)	83,60 ± 2,30^{a}	41,60 ± 2,07^{bc}	67,20 ± 1,92^{ab}	70,40 ± 3,36^{ab}	51,00 ± 2,92^{b}
Concentration (x10^{6}/ mL)	83,00 ± 2,55^{a}	50,00 ± 2,24^{ab}	72,20 ± 1,48^{b}	77,20 ± 1,92^{bc}	65,80 ± 2,78^{c}
Testosteron level (nmol/L)	13.68 ± 0.65^{a}	8.60 ± 0.40	10.90 ± 0.57	12.89 ± 0.85^{a}	9.69 ± 0.42
Testicular weight (mg)	306 ± 20,73^{a}	216 ± 20,73^{ab}	258 ± 23,76^{b}	292 ± 23,88^{a}	245 ± 7,07^{ab,b}
Epididymal weight (mg)	10,84 ± 0,39^{a}	7,68 ± 0,40	10,02 ± 0,58^{ab}	10,36 ± 0,63^{a,ab}	8,46 ± 0,34

Data are presented as mean ± SD. Differences in superscripts in the same rows show significant differences (p<0,05). K1 (negative control): Na-CMC 0,5%; K2 (positive control): S. aureus 0,5 Mc Farland; P1 (treatment group 1): S. aureus 0,5 Mc Farland + C. nardus 25 mg/kg BW; P2 (treatment group 2): S. aureus 0,5 Mc Farland + C. nardus 50 mg/kg BW; P3 (treatment group 3): S. aureus 0,5 Mc Farland + C. nardus 100 mg/kg BW.

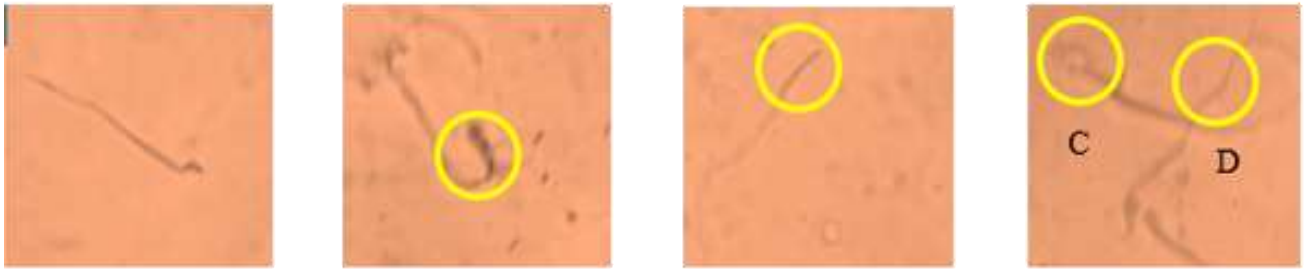

Fig.1. Sperm cell morphology in adult mice had normal (N) and abnormal (A: twisted midpiece or tail; B: head malformation (like a needle); C: larger head; D: broken tail).

Table 2. Effect of *C. nardus* extract on spermatogenic cells and diameter of the seminiferous tubule in mice infected by *S. aureus*

Parameters	K1	K2	P1	P2	P3
DST	216,52 ± 16.65[a]	105.60 ± 13.91[b]	141.41 ± 13.19	186.03 ± 29.70[a]	115.79 ± 13.85[b]
Spermatogonia cells	82,80 ± 4,38[a]	60,00 ± 4,00	75,20 ± 4,60[ab]	80,00 ± 3,16[a,ab]	67,20 ± 4,15
Spermatocytes cells	99,60 ± 5,73[a]	65,20 ± 7,95	87,80 ± 6,72[ab]	98,00 ± 6,33[a]	81,20 ± 3,35[ab]
Spermatid cells	284,0 ± 42,9[a]	179,2 ± 18,6	255,2 ± 39,5[a,ab]	268,0 ± 24,5[a,ab]	204,8 ± 15,1

Data are presented as mean ± SD

Differences in superscripts in the same rows show significant differences ($p<0.05$). K1 (negative control): Na-CMC 0,5%; K2 (positive control): *S. aureus* 0,5 Mc Farland; P1 (treatment group 1): *S. aureus* 0,5 Mc Farland + *C. nardus* 25 mg/kg BW; P2 (treatment group 2): *S. aureus* 0,5 Mc Farland + *C. nardus* 50 mg/kg BW; P3 (treatment group 3): *S. aureus* 0,5 Mc Farland + *C. nardus* 100 mg/kg BW. DST = Diameter of Seminiferous Tubules.

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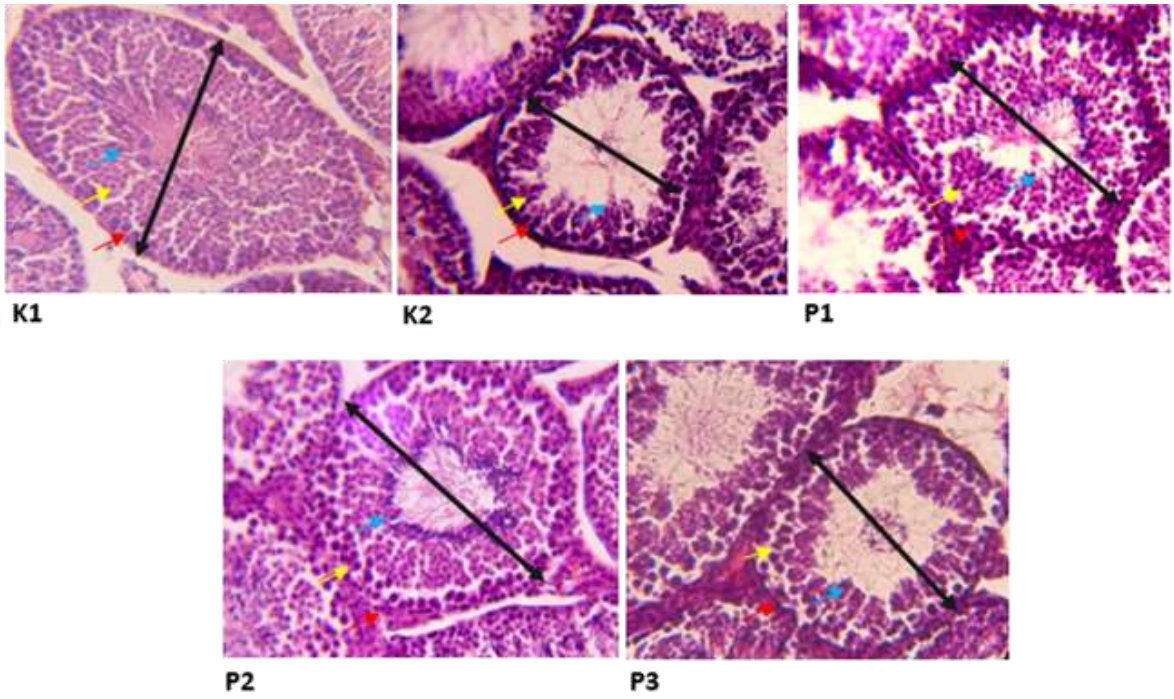

Fig. 2: Testicular seminiferous tubules in different groups. Red arrows indicate spermatogonia cells, yellow spermatocytes, blue spermatids, and black seminiferous tubule diameter. (Hematoxylin Eosin: 400x).

DISCUSSION

In ethnomedicines, the use of medicinal plants, commonly known as medicinal herbs, has been suggested for the treatment of a wide variety of disorders at various points in history and continued to be practiced today. Throughout the history of humanity, people have relied on the therapeutic properties of various plants to heal a variety of illnesses[21,22]. New scientific approaches have been integrated into conventional medical practice to facilitate the standardization of phytotherapy, which refers to the use of numerous plant-based remedies in combination with one another[23,14].

According to this study, The decreased quality of spermatozoa that has been caused by bacterial infection was partially restored by the use of an extract of *C. nardus*. There was a statistically significant difference ($p<0.05$) between the group that was treated with *C. nardus* extract and the group that served as the positive control and was treated with *S. aureus*. To acquire the optimal results in terms of epididymal and testicular weight, sperm motility, normal morphology of sperm, testosterone level, sperm concentration, and the number of spermatogenic cells, a dose of 25 mg/kg of body weight was shown to be the most effective. The increased dose that was administered did not result in an improvement in the quality of the spermatozoa. Even at a dose of 100 mg/kg of body weight, the quality of sperm that had been infected with *S. aureus* was not improved.

S. aureus is a pathogen bacteria that are typically found in the female genital tract[2]. This bacteria is known to have a toxic effect on the epididymal duct in addition to disrupting the process of spermatogenesis and also could well interact with the CD14 on the surface of the sperm,

primarily in the head and tail region[24]. This interaction will result in increased levels of sperm membrane lipid peroxidation, production of reactive oxygen species, and caspase-mediated apoptosis in the subsequent stages[6,10,25]. A reduction in the motility of spermatozoa and an increase in lipid peroxidation are both indicators that cell damage has been caused *by S. aureus*[26,16,27]

The process of male reproduction is a complicated one that requires a great deal of energy. The hypothalamus and the pituitary gland use a variety of feedback regulation mechanisms to control the function of the testis, which serves as the primary organ of the male germline[1,28]. GnRH, FSH, and LH are the primary hormones responsible for controlling regulatory mechanisms via their actions on the hypothalamus-pituitary-testis axis[29]. Through neurons that secrete GnRH, the hypothalamus can monitor the condition of the reproductive system. Through the anterior pituitary, GnRH stimulates the release of LH as well as FSH. LH stimulates the production of testosterone by acting on testicular interstitial cells and increasing their permeability. FSH is the hormone that is responsible for the stimulation of the seminiferous tubules, which is necessary to maintain sperm production[30].

A decrease in the amount of testosterone in the body can lead to reproductive disorders. Testosterone is a hormone that is primarily found in men[31]. The precise and coordinated secretion of GnRH, LH, FSH, and testosterone is what regulates male reproductive function. Testosterone also plays a role in this regulation. The production of spermatozoa as well as the synthesis and release of testosterone are both the responsibility of the testes[32]. A lower testosterone level may be associated with a more complex condition, such as erectile dysfunction[33,34]. Phosphodiesterase 5 (PDE-5) inhibitors, such as Sildenafil, Tadalafil, and others, are typically prescribed to patients who have been diagnosed with erectile dysfunction. According to several studies, the effectiveness of PDE-5 inhibitors is improved when there is an adequate quantity of testosterone in the body[35]. Erectile dysfunction may be caused by several causes, but the one that is caused by a lack of testosterone is likely treatable by using medicines that raise testosterone levels[33]. Other causes of erectile dysfunction may not be so easily remedied. In the current study, after administering a treatment consisting of *C. nardus* extract for a period of 14 days, significant increases in testosterone levels were observed in the serum.

One of the herbal medications that have been shown in a recent study to have the potential impact of protecting organs against free radicals is called *C. nardus*[36,19]. Antioxidants, flavonoids, phenolics, and terpenoids are the types of phytochemical substances that can be found in *C. nardus*[37,38]. Antioxidants shield lipid membranes against damage caused by superoxide, ammonium, and peroxide radicals, which are each capable of causing cellular degeneration[39,40]. Antioxidants also participate in the electron transport process that occurs during the final step of energy production in mitochondria[41]. To improve sperm motility, mitochondria must produce ATP or other forms of energy before fertilization may proceed[42].

Fertilization is dependent on several factors, one of which is motility. If motility were to suffer any kind of impairment, this would have a negative influence on fertilization capacity[43]. During their journey through the epididymis, sperm develop the ability to move about, and the epididymis is responsible for the initialization of this ability by both providing a distinct microenvironment and also secreting proteins that are essential for the initialization of sperm motility[21,44].

In the previous research, the protective effect of lemongrass in male reproduction after being triggered by hydrogen peroxide was described. And the results demonstrated that an increase in testosterone level, sperm characteristics, testicular and epididymal weight, as well as a reduction in serum and tissue homogenate *malondialdehyde* (MDA) and testicular histopathological changes, can be brought about by administering 100 mg of lemongrass extract per kg of body weight[45]. In correlation with the previous research, we found that *C. nardus* extract could improve sperm quality in mice after being induced by *S. aureus*. The potential dose of *C. nardus* that could improve sperm quality significantly was 25 mg/kg of body weight, and this was found to be the optimal amount for this improvement.

CONCLUSION

The *C. nardus* extract protects spermatozoa cells by repairing the sperm quality decrease caused by *S. aureus* infection. A low to a middle dose of *C. nardus* extract provides more protection than a high dose. A high dose of *C. nardus* extract is ineffective at improving sperm motility and concentration.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this research.

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A AND V PUBLICATIONS

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To begin with, this manuscript is acceptable based on the Turnitin plagiarism check. In addition, in order to improve the readability of the paper by an English editing servi
Furthermore, the paper is easy to follow by the reader. However, I may require some comments on the following issues.
Title: The title is good.
Abstract: This section was well-written and easy to understand.
Introduction: Adequate.
Material and Methods: Okay
Results: Good
Discussion: The discussion of the study lacks information about the important topics.
Conclusion: Good.
References: Updated references are needed.

Arif Nur Muhammad Ansori

- Is the work clearly and accurately presented and does it cite the current literature? Yes
- Is the study design appropriate and is the work technically sound? Yes
- Are the conclusions drawn adequately supported by the results? Yes
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