Original Article

Essential oil extracted from plant tuber of nutgrass "*Cyperus rotundus*" effectively decreased sperm quality of mice

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ABSTRACT

Tuber extract of nutgrass (*Cyperus rotundus* or rumput teki), induce apoptosis in leukemia cell lines. This effect related to the essential oil content of nutgrass. The present study aimed to evaluate the effect of nutgrass essential oil on the quality of sperm in mice. Mice were divided into 4 groups using a completely randomized design. Group 1 (control) received only distilled water. Groups 2, 3, and 4 received 9, 18 and 36 mg/ 30gBW essential oil, respectively for 35 days. At the end of 35 days, the total number of sperms was 51, 25.16, 16.66, and 11.83 million/ mL, and the sperm motility was 75.5%, 55.66%, 32.66% and 23.33%, in group 1, 2, 3, and 4 respectively. The results showed that the nutgrass essential oil in a dose-dependent manner decreased the motility and number of sperms in mice. It is suggested that the essential oil extracted from the *Cyperus rotundus* tuber has the potential to be utilized as an antifertility agent.

Keywords: Sperm quality, Cyperus rotundus, Nutgrass, Rumput teki, Essential oil.

Introduction

The plants have many applications due to their different components [1-3]. The active ingredients found in purple nutsedge tuber are sesquiterpenes including isokobusone, kobusone, sugeonol, patchoulenone, cyperotundone, cyperene, β-selinene, and α-cyperone. In addition to these active components, the purple nutsedge tuber also contains several chemicals, namely saponins, glycosides, starches, tannins, flavonoids, and alkaloids [4, 5] which can be used as antibacterial, menstrual, laxative, and contraceptives. Contraceptives affect the reproductive process, in this case, by inhibiting the ovulation of the menstrual cycle in humans and the estrous cycle in mice (*Mus musculus* L.), inhibiting sperm penetration, and embryo implantation and fertilization so that the pregnancy process is difficult to occur and if it does occur then the fetus will possibly experience disability [6].

Infertility in men can be determined through the measurement of concentration, motility, and morphology of sperm. A decreased motility and concentration of sperm, associated with the disability of the secretion function of Sertoli cells and Leydig cells, causes imperfections in the process of spermatogenesis and sperm maturation in the epididymis, leading to decreased motility, and increased morphologic abnormalities of sperm [7]. The number of spermatogenic cells is the number of spermatogonia cells, primary spermatocytes, secondary spermatocytes, spermatids, and sperm which are located in the seminiferous tubules that indicate the process of spermatogenesis that occurs in the testes. The number of spermatogenesis cells is the number of spermatid and sperm cells. The time required for the formation of spermatogonia is 3 days, primary spermatocytes for 16 days, secondary spermatocytes for 26 days, spermatids for 36 days, and sperm for 49 days [8]. Sperms formed in the testes are channeled into the epididymis to undergo maturation.

Sperm motility is often used as an indicator of sperm fertility. Motility testing is performed to determine the movement of sperm. However, the movement of sperm is also influenced by the integrity of the morphological structure of sperm. The percentage of motility is the percentage of sperms that move

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progressively forward [9]. Cell plasma membrane that is still intact will affect organelles in cells [10, 11]. This causes sperm to move progressively and stay alive (viable) so that they are able to fertilize [10, 11].

Disruption of spermatogenesis through cytotoxic testicular mechanisms causes a decreased number of sperm, increased number of abnormal sperms, and decreased sperm motility [12]. Decreased sperm motility with incubation length is thought to be due to sperm running out of energy. Energy is needed by sperm to actively move and is obtained from the decomposition of ATP into ADP and AMP in the mitochondria that are inside the tail of sperm [13, 14]. If ATP and ADP are depleted, the contraction of fibrils from the tail of the sperm will stop so that the sperm do not move. To maintain the continuity of sperm motility, ATP and ADP must be formed again in the form of alternating reactions [15]. Provision of essential oils of purple nutsedge grass tubers in animals, it is necessary to know whether their essential oils can decrease the number and motility of sperm of mice (*Mus musculus* L.).

Materials and Methods

Preparing essential oils of nutsedge tubers

The study began with identifying and determining purple nutsedge plants based on the observations of plant morphological characteristics such as tubers, roots, stems, leaves, and flowers at the Botanical Laboratory of the Department FMIPA University of Lampung. The tubers were washed with water, dried at room temperature for about 7 days, and cut to small sizes. A total of 1000g of tubers of purple nutsedge were mixed with d distilled water (2/3 the flask contents) and heated at 100°C for 3 h until a mixture of water and oil was obtained (until essential oil drops were not formed). Next, the oil mixture was evaporated in a vacuum at a low temperature until essential oil was obtained. Finally, an essential oil that was still mixed with a little water was separated by the addition of $MgSO_4.7H_2O$ until saturated. The essential oil obtained was used as the sample for further research.

Determination of the dose of the nutsedge tuber essential oil

The experimental animals used were 24 male mice (*Mus musculus* L.) with an average body weight of 25-35g. The essential oils were administered orally to the animals with the following dosages:

1. Group 1 (Control): mice treated with 0.3 ml of aquabides over 35 days.
2. Group 2: mice were treated with essential oils at a dose of 9 mg/30g BW in 0.3ml aquabides for 35 days.
3. Group 3: mice were treated with essential oils at a dose of 18 mg/30g BW in 0.3ml aquabides for 35 days.
4. Group 4: mice were treated with essential oil with a dose of 36 mg/30g BW in 0.3ml aquabides for 35 days.

Treating experimental animals with essential oils of purple nutsedge tubers

Experimental animals were weighed first, kept in controlled laboratory conditions by feeding them *ad libitum*. Treatment with essential oils of nutsedge tubers was done orally using a special syringe whose edges were blunted and given a small rubber pipe. The essential oils were administered to experimental animals by adjusting the volume of suspension with body weight. The treatment was given for 35 days based on the spermatogenic cycle of mice.

Calculation of the number of sperm

The number of sperms was calculated using the Improved Neubauer booth (hemocytometer). Suspension of sperm was diluted in NaCl solution 0.9%, dropped onto a counting chamber (hemocytometer), and then covered with a cover glass. The number of sperms was counted under a light microscope (400x). After knowing the number of sperms, their concentration was calculated using the following formula:

$$\text{Concentration (million/ml)} = \text{Dilution factor} \times \text{Count in 5 squares} \times 0.05 \times 10^6 \quad (1)$$

Calculation of sperm motility

After finishing treatment, mice were sacrificed and dissected. Sperms were taken from the epididymis. One of the epididymides was placed on a watch glass that contained 0.9% NaCl, then the organs were chopped into small pieces. A drop of sperm suspension in 0.9% NaCl was dropped on a hemocytometer, covered with a glass cover, and observed under a microscope (400x). The percentage of the number of motile sperms was calculated by dividing the number of moving sperms by the number of observed sperms and multiplied by 100%.

Statistical Analysis

Data analysis was performed using one-way ANOVA and LSD in post hoc test, both ANOVA analysis and LSD test were using $\alpha \leq 0.05$ as the significant criterion.

Results and Discussion

The number of sperms

The results of the effect of tuber essential oils on the number of sperms of mice are shown **Table 1**.

Table 1. Sperm counts of mice after treating with essential oils extracted from tubers of nutgrass

Treatments	Sperm counts (million/ml) (mean ± SD)
Control	51.00 ± 8.02[a]
9 mg / 30g BW	25.16 ± 2.85[b]
18 mg / 30g BW	16.66 ± 2.33[c]
36 mg / 30g BW	11.83 ± 2.32[c]

Values followed by the same superscript were not statistically different at α = 0.05.

Sperm Motility

The results of the effect of purple nutsedge tuber essential oils on the motility of sperm of male mice can be seen in **Table 2**.

Table 2. Sperm motility of mice after treating with essential oils extracted from tubers of nutgrass

Treatments	Sperm motility (%) (mean ± SD)
Control	75.50 ± 9.91[a]
9 mg/30g BW	55.66 ± 10.42[b]
18 mg/30g BW	32.66 ± 16.83[c]
36 mg/30g BW	23.33 ± 11.82[c]

Values followed by the same superscript were not statistically different at α = 0.05.

The results of the effect of purple nutsedge tuber essential oils on the number of sperms of male mice revealed that there were significant differences in the average number of sperms between the control and treatment groups. The control group had the highest average number of sperms, while the treatment group, especially T3, had the lowest number of sperms. The results showed that the number of sperms (million/ml) in male mice that were orally administered with 0.3 ml/day essential oils of purple nutsedge tubers for 35 days was decreased compared to the control group. In control mice, the average number of sperms was 51.00 million cells/ml; this number is the normal amount. In T1, the average normal number of sperms was 25.16 million cells/ml, whereas, in T2 and T3, the average number of sperms was less than 20 million per ml of cement which was 16.66 and 11.83 million cells/ml and includes light oligozoospermia light (less normal).

The process of sperm formation or spermatogenesis in male animals occurs when puberty has begun. During this time, the hypothalamus hormones and the pituitary glands actively control reproductive hormones. This causes the testicles to enlarge and develop due to seminiferous tubular activity, and Leydig cells begin to produce the hormone testosterone. Spermatogenesis occurs in the testicular seminiferous tubules and involves spermatogenic cells, Sertoli cells, pituitary hormones, and testosterone.

The results showed that the essential oil of purple nutsedge tubers decreased the number of sperms. This effect causes the number of sperms to be lower with increasing doses of essential oil treatment. The lower number of sperm is thought to occur because the active ingredients of essential oils affect the components involved in spermatogenesis. It is well known that Sertoli cells are responsible for the maturation of spermatogenic cells in the seminiferous tubules, such as for the supply of nutrients and hormones for the maturation of sperms. The presence of a rough endoplasmic reticulum, a number of mitochondria and Golgi bodies in the basal cytoplasm of Sertoli cells characterizes Sertoli cells for protein metabolic activity, such as Androgen Binding Protein (ABP) and steroid biosynthesis [16].

No increase in the number of sperms at the reversibility stage was observed; it was decreased or was less when compared to the number of sperms taken at the end of the treatment. It is estimated that this happens in the following ways: For normal spermatogenesis after stopping the treatment for more than 40 days. This is consistent with the results of Hess and Chen (1992), which showed that 90 days after the treatment was stopped, the sperm count was lower than the sperm count in the control group mice [17].

It can be further explained that testosterone is needed to begin the process of the first meiosis, namely the formation of primary spermatocyte cells into secondary spermatocyte cells. Al-Makhzoomi (2008) states that testosterone plays a role in the division of the first meiotic prophase that causes a decrease in the number of secondary spermatocytes [18]. This is also supported by Johnson's statement (2018) that spermatocytes are very sensitive to external influences and tend to suffer damage after the first prophylactic meiosis, especially at the pakiten stage, i.e. when crossing between homologous chromosomes [19]. At this stage, the nucleus and cytoplasm grow into the largest cells among the spermatogenic cell layers, when spermatocytes suffer damage such as tubular atrophy, tubular necrosis, and loss of intermedia cells. Cummins *et al.*, (1994) stated that this causes spermatogenic cells to degenerate and phagocytosis by Sertoli cells so that the number of spermatocytes is reduced [20]. Decreasing the number of spermatocytes causes the number of spermatids to also decrease because spermatocytes that have second meiosis will become spermatids. According to Sengupta et al (2019), the testosterone hormone will maintain all stages of the development of spermatids [21]. A decrease in the hormone testosterone results in the release of spermatids from the Sertoli cells into the seminiferous tubule lumen which causes the failure of spermiogenesis [22] or it is suspected that because the number of spermatids decreases it will also decrease the number of sperms produced, because spermatids will experience spermiogenesis to become sperm [23].

The average percentage of motile sperms of male mice was decreased (in the treatments with essential oils of purple nutsedge tubers with a dose of 9 mg/30gBW, 18 mg/30gBW, and 36 mg/30gBW they were 55.66%, 32.66%, and 23.33% respectively). While the percentage of sperm motility of male mice that were not given essential oils of purple nutsedge tubers (control) was 75.50%. After statistical tests, the results revealed that the control treatments were significantly different

from groups 2, 3, and 4 (<0.05). Treatment of 9 mg/30g BW, was significantly different from 18 mg/30gBW, and 36 mg/30gBW (<0.05). Whereas 18 mg/30gBW treatment was not significantly different from the 36 mg/30gBW treatment (P = 0.213).

Sperm motility can occur if sperm have mitochondria that function well to produce mobile energy through oxidative phosphorylation [24]. It is suspected that the active compounds contained in the purple nutsedge can inhibit the function of mitochondria in producing energy so that the percentage of sperm motility after administration of essential oils of the purple nutsedge tubers decreases. The oxidative phosphorylation process requires the ATP synthase enzyme. It is suspected that the active compounds contained in the purple nutsedge can inhibit the activity of the ATP synthase enzyme so that the available energy cannot be used and causes the sperm to be immobile.

Sperm motility shows the movement of sperm by the presence of flagella that gets energy from the neck of the sperm which is rich in mitochondria. The results of research on the motility of sperm show that papaya seed extract greatly influences motility. Sperms that are released from the testes into the epididymis are not mature because they are not yet motile and cannot be used to fertilize the ovum. The maturation process of sperm occurs in the epididymis.

According to Lohiya *et al.* (2005), decreased sperm motility is not caused by the stage of maturation in the epididymis but has already occurred while still in the testis [25]. Sperm motility can occur if sperms have mitochondria that function well to produce mobile energy through oxidative phosphorylation. Januskauskas and Zillinskas, (2002) added that the thing that plays a role in determining sperm motility is the stage of spermiogenesis [24]. If during the metamorphosis of spermatids, sperms become abnormal (such as decondensation in the nucleus), the formation of acrosomes and mitochondria will affect the success of motility in the epididymis.

Sperm can become motile because of the energy from the neck that is distributed to the tail. This part causes sperm to move forward. So, the main key to the movement of sperm is the production of energy by the mitochondria in the neck of the sperm. It has been suggested that the essential oil of the tubers causes organelle abnormalities in the neck of the sperm, namely vacuolization of the mitochondria and structural abnormalities in the form of a bent neck [26]. Thus, the mitochondrial function in producing energy is not optimal and ultimately affects the motility of sperm. Lohiya *et al.* (2005) stated that this affects directly the mechanism of sperm formation, while the effect on the epididymis is indirect. Furthermore, it is proved by the correlation between the number and motility of sperm. The number of sperm in the treatment group was less when compared to the number of sperm in the control group. Thus, the essential oil of the tubers affects spermatogenesis at the stage of the metamorphosis of spermatids into sperm. If the spermatozoa produced are abnormal in their formation, of course, this will also affect the maturation of the epididymis.

Menezo *et al.* (2010) reported that sperm motility can occur due to contractions of fibrils in the tail of sperm [27]. This can happen if sperms have energy in the form of ATP. ATP is produced from simple sugar metabolism through respiration. Respiration can occur if sperm gets nutritional intake from outside the cell. Spermatozoa motility occurs because of the movement of the flagellum which consists of microtubules. Flagel motion is the sliding motion between doublets mediated by dynein. Dynein is a protein that has a group that is responsible for ATPase, which contributes to the hydrolysis of ATP. Dynein performs a movement cycle because of the ATP produced by the mitochondria. ATP is activated by the ATPase enzyme to release the first phosphate bonds to form ADP and inorganic phosphate by releasing energy for fibril contraction. If the supply of phosphate, sperm contraction of the fibrils will stop and motility also stops.

Conclusion

The essential oil extracted from the tuber of nutgrass (*Cyperus rotundus*) has been revealed to reduce the parameters of sperm quality in male mice in a dose-dependent manner. It can be concluded, therefore, that the essential oil extracted from the tuber of *Cyperus rotundus* has the potential to be used as an anti-fertility agent.

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