



EFFECTIVENESS OF BLACK Cumin (*Nigella sativa* L.) EXTRACT ON INCREASING STAMINA IN MALE MICE (*Mus musculus*)

EFEKTIVITAS EKSTRAK JINTEN HITAM (*Nigella sativa* L.) TERHADAP PENINGKATAN STAMINA PADA MENCIT JANTAN (*Mus musculus*)

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Abstract

Research using ethanol extract from black cumin seeds aims to find out whether black cumin seed extract (*Nigella sativa*) can be effective in increasing the stamina of male mice. The research design used was a completely randomized design (RAL). The samples used were 24 male mice consisting of four treatments and six repetitions. The dosing treatment consisted of a normal control group (P0), 30 mg/kg weight (P1), 60 mg/kg weight (P2), and 120 mg/kg weight (P3). The swimming test method was used to determine the effect of increasing stamina on mice. Data on the swimming duration of mice were analyzed using the ANOVA test then continued using the Post Hoc Test, namely LSD. The average stamina of mice based on swimming duration was the highest, namely at a dose of 120 mg/kgW (P3). Administration of black cumin seed extract (*Nigella sativa*) at doses of 30 mg/kgBW, 60 mg/kgW, and 120 mg/kgW for 14 days provided effectiveness in increasing stamina in male white mice (*Mus musculus*) as indicated by an increase in the swimming duration of the mice in each treatment.

Keywords: Ethanol extract; *Mus musculus*; *Nigella sativa*

Abstrak

Penelitian menggunakan ekstrak etanol biji jinten hitam bertujuan untuk mengetahui apakah ekstrak biji jinten hitam (*Nigella sativa*) dapat berpengaruh terhadap peningkatan stamina mencit jantan. Rancangan penelitian yang digunakan ialah rancangan acak lengkap (RAL). Sampel yang digunakan sebanyak 24 ekor mencit jantan yang terdiri dari empat perlakuan dan enam pengulangan. Perlakuan pemberian dosis terdiri atas kelompok kontrol normal (P0), 30 mg/kgBB (P1), 60 mg/kgBB (P2), dan 120 mg/kgBB (P3). Metode swimming test digunakan untuk mengetahui efek peningkatan stamina terhadap mencit. Data durasi renang mencit dianalisis menggunakan uji ANOVA kemudian dilanjutkan menggunakan uji Post Hoc Test yaitu LSD. Rata-rata stamina mencit berdasarkan durasi renang yang paling tinggi yaitu pada dosis 120 mg/kgBB (P3). Pemberian ekstrak biji jinten hitam (*Nigella sativa*) pada dosis 30 mg/kgBB, 60 mg/kgBB, dan 120 mg/kgBB selama 14 hari dapat berpengaruh dalam peningkatan stamina pada mencit putih jantan (*Mus musculus*) ditandai meningkatnya durasi renang mencit dimasing-masing perlakuan.

Kata Kunci: Ekstrak etanol; *Mus musculus*; *Nigella sativa*

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INTRODUCTION

Health-related components of physical fitness can be categorized as endurance or stamina, flexibility, balance, agility, and coordination, and endurance/stamina is standardized as cardiorespiratory endurance, muscle endurance, and muscle strength (Haskel & Kiernan, 2000). Great efforts are made by athletes, trainers or sedentary people to earn better endurance capacity, while endurance capacity is also important to endurance athletes as well very important to common individuals. Therefore, improvement of the overall endurance will also benefit the physical fitness of common people.

All movements of the human body require energy. Cells use material energy through adenosine triphosphate (ATP), which is called energy currency (Imamura et al., 2009). The continuous supply of ATP to the processes that support skeletal muscle contraction during exercise is critical to exercise performance lasting seconds to hours (Hargreaves & Spriet, 2020). Intensity and duration of exercise are major determinants affecting the relative contributions of energy production pathways during exercise (Hargreaves, 2022). So that we get good stamina, we should exercise with an appropriate and continuous frequency, intensity, time, and type of exercise (Huang et al., 2022).

Exercise is one of the activities carried out to improve and maintain the body. It remains healthy and stable. Sport is a very important activity to maintain health and physical fitness. Physical fitness cannot be separated from daily movement activities which are caused by the organs and muscles in the body moving every day. The goal is to improve the physiology of power and increase muscle stamina, muscle strength, cardiorespiratory endurance, and flexibility (Moctar et al., 2021). This occurs due to the ability of the systems in the body to create the quality of life in every physical activity, such as carrying out an activity without causing excessive fatigue (Gemi et al., 2019).

Fatigue is always associated with a weakening of a person's ability to create a reaction to a stimulus. In general, fatigue is often referred to as tiredness. Tiredness is a condition when muscle cells are no longer able to contract, causing a decrease in the efficiency of the activities carried out (Parwata, 2015). This condition is caused by strong and prolonged muscle contractions resulting in the inability of the muscle fiber contraction process to continue to provide adequate work results. the same as before (Wahyuni & Indriyani, 2019).

People continue to look for ways to maximize physical activity to achieve desired goals and slow down fatigue. One of them is consuming drinks that keep the body fresh, such as using herbal plants. Indonesia is a country that has many kinds of biodiversity, both animals and plants. Plants in Indonesia have an average of 3,000 types of herbal plant potential, of which 340 species have medicinal properties. Herbal plants have many properties for curing various diseases and increasing stamina (Anaba et al., 2021).

The use of herbal plants is currently in great demand among the public because of easy to obtain, economical, and as an alternative treatment. Herbal plants have increased because they are more affordable in terms of price and availability of ingredients (Darmawati, 2017). Along with the increasing use of chemical-based stamina-enhancing drugs containing caffeine among the public, it is necessary to balance this with efforts to develop stamina-enhancing drugs from plants with herbal properties (Mafitri & Parmadi, 2018).

One of the herbal plants that is often used by Indonesians is the black cumin plant (*Nigella sativa*). This black cumin has many benefits for humans, as in the interpretation written in the hadith history, Rasulullah SAW said that "Indeed, in the Habbatussauda plant (Black cumin) there is a cure for all kinds of diseases, without exception of death" (HR. Bukhori and Muslim). Black cumin (*Nigella sativa*) is a herbal plant that has many benefits for curing diseases. The biological activity of black cumin seeds is antibacterial, anti-inflammatory, antioxidant, and immunostimulant (Mahfur, 2018).

The main compounds contained in the black cumin plant are thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine, and alpha-hedrin. Tymoquinone is an anti-allergic, and anti-inflammatory, and can also improve the immune system (Hasanah et al., 2019). Black cumin (*Nigella sativa*) is not only proven to be a healing medicine but also contains more than 100 elements that support the human immune

system (Kurniasih et al., 2022). Therefore, black cumin is thought to have the effectiveness of increasing stamina which can increase physical activity, so to prove this, research needs to be carried out to test the effectiveness of increasing stamina from black cumin seed extract (*Nigella sativa*).

In this research, mice (*Mus musculus*) were also determined to be studied. This is because mice have long served as the preferred species for research animal models due to their anatomical, physiological, and genetic similarity to humans. Advantages of rodents include their small size, ease of maintenance, active, short life cycle, and abundant genetic resources. The age taken for mice is around 2–3 months old or old enough, body length of 10–15 cm, healthy, active, agile, and without defects (Mutiarahmi et al., 2021).

MATERIALS AND METHODS

This research was carried out from January until March 2023 at the Integrated Laboratory of UIN Raden Fatah Palembang, Ecology Lab Room 309, and Animal House Abdul Muhih Palembang. This type of research is quantitative experimental research. The research design used was a completely randomized design (RAL).

The tools and materials used in this research were mouse cages, a place for mice to eat and drink, an aquarium (swimming place) with a height of 18 cm, analytical balance, oven, incubator, 250 mL beaker, jam bottle, injection, blender, gastric sonde and 1 mL syringe, 10 mL measuring cup, syrup bottle, glass jar, stirrer, filter paper, glass funnel, 50 mL measuring flask, watch glass, vial bottle and rotary evaporator. Meanwhile, the materials used during this research included 24 male Swiss Webster mice (*Mus musculus*), distilled water, black cumin (*Nigella sativa*), feed given twice/day, tween 80, and 96% ethanol.

Put 300 g of black cumin seeds (*Nigella sativa*) into a maceration container, then add 3 L of 96% ethanol until totally submerged. Store and place the container at room temperature where it is not exposed to direct sunlight for 3×24 hours, stirring occasionally. It was filtered using filter paper. The mass obtained was evaporated using a rotary evaporator at a temperature of 50 °C with a speed of 60 rpm. Next, the extract obtained was evaporated using an incubator until it became a thick extract. The thick extract was put into a vial and the total extract was weighed until it became a thick extract (Hidayat et al., 2022).

The test animals used were 24 adult male Swiss Webster strain mice (*Mus musculus*) aged 3–4 months with a body weight of around 20–35 g, healthy and without defects. The test animals were divided into four groups consisting of the normal control group (P0), the 30 mg/kgW dose group (P1), the 60 mg/kgW dose group (P2), and the 120 mg/kgW dose group (P3) with six repetitions. Before being given treatment, all mice were weighed, and their body weight was recorded. The treatment was given to mice orally using a gastric probe. The volume of the dose solution given is 0.2 mL for every 20 g of body weight of mice (standard). Treatment was given once a day for two weeks (Mao-ye & Li-guo, 2011). The mice were treated using the swimming endurance test method. A weight equivalent to body weight was attached to the root of the mouse tail by lead fish sinkers. The tank was maintained at 28 °C during the swimming process and the endurance for each mouse was measured as swimming time recorded from the beginning of the time to exhaustion, defined by observing uncoordinated movements and failure to return to the surface. The time of floating, struggling, and making necessary movements was assessed until possible drowning and exhaustion. The other details of the exhaustive swimming exercise were previously described (Huang, 2022).

RESULTS

In this research process, experimental animals were carried out starting from animal acclimatization for 7 days. After that, place the mice in an aquarium with a water level of 18 cm. It started with the test animals being acclimatized for seven days by training them to swim on the 7th day. All groups P0, P1, P2, and P3 were recorded for the time they showed signs of fatigue because multiple environmental conditions influence the behavior of mice and it was important to ensure wide utility of the selected fatigue measures, methods were selected that would allow spontaneous behavior

of the animals before being given treatment. On day 8, the test animals were treated with a dose of black cumin extract from each group for two weeks.

After two weeks, the test animals were re-swimming by placed in the aquarium until signs of fatigue appeared when the mice failed to rise to the surface of the water to breathe within 5 seconds by leaving their heads below the surface of the water. The parameters measured are that the test animal does not move its legs to swim, its body is perpendicular with its tail not moving. It leaves its head below the water surface for 5 seconds. The data that has been obtained is then collected and analyzed using statistical tests. Each group was treated for two weeks. The results of testing the stamina of mice using a swimming test showed a difference in the increase in the average swimming duration of mice.

Figure 1 shows that there is a difference in the average amount of time the mice's stamina increases from each treatment given. The increase in stamina before treatment is in the range of 6–7 minutes. After being given treatment (being fed black cumin seed extract), stamina increased, which was in the range of 3–25 minutes in treatments P1 (30 mg), P2 (60 mg), and P3 (120 mg) ($P=0.000$, mean \pm SD, 11.56 ± 4.6).

Based on the increase in stamina where there was an increase in time for each dose, analysis was carried out using SPSS 22.00 for Windows to test the hypothesis regarding the effectiveness of black cumin extract on increasing the stamina of mice. The first data analysis is to assess whether the data is normally distributed or not. Data distribution was analyzed using the Shapiro-Wilk normality test because the sample size was small ($n < 50$).

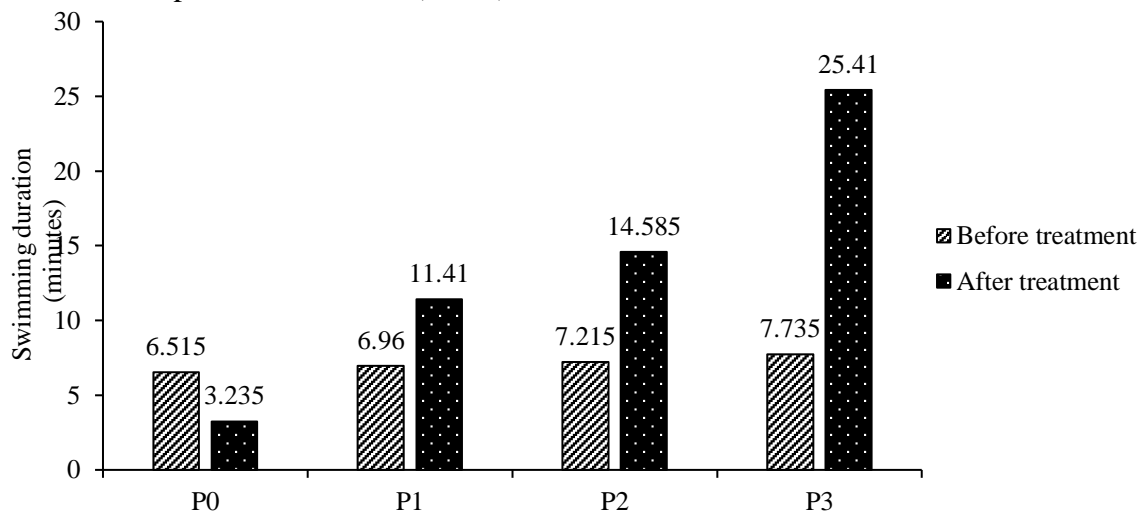


Figure 1. Average stamina of mice normal control group (P0), 30 mg/kg weight (P1), 60 mg/kg weight (P2), and 120 mg/kg weight (P3)

Based on the table of normality test calculation results, the significance value (p) is above the predetermined degree of significance ($P > 0.05$). In Table 1, data from the stamina group before treatment and stamina after treatment showed that the average significance value was above the predetermined degree of significance ($P > 0.05$). Next, a homogeneity test was carried out to determine whether the data was normally distributed or not. The homogeneity test results can be seen in Table 2.

Table 1. Stamina homogeneity test results before and after treatment

	Treatment	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Before treatment	P0	.243	6	.200*	.866	6	.210
	P1	.293	6	.117	.854	6	.169
	P2	.291	6	.122	.843	6	.137
	P3	.238	6	.200*	.941	6	.664
After treatment	P0	.251	6	.200*	.879	6	.263
	P1	.239	6	.200*	.861	6	.194
	P2	.283	6	.144	.856	6	.175
	P3	.176	6	.200*	.983	6	.964

Table 2. Shapiro normality test results wilk increased stamina before and after treatment

	Levene statistic	df1	df2	Sig.
Before treatment	.950	3	20	.435
After treatment	2.713	3	20	.072

Table 2 shows that for data on stamina values before treatment and stamina after treatment, significance values were obtained above the predetermined degree of significance ($P > 0.05$). This indicates that the two groups of data come from populations with the same variance (homogeneous). After obtaining that the two groups of data were homogeneous, a statistical comparative analysis was carried out using One Way Anova parametric statistics.

One Way Anova test shows $P = 0,000$ ($P < 0.05$), so H_0 is accepted, so it can be concluded that the hypothesis has been proven correct that there is a difference in the effectiveness of increasing stamina in black cumin seed extract (*Nigella sativa*). Next, to find out which groups had significant differences, a Post Hoc test was carried out. The results of the Post Hoc LSD test can be explained that in the group data after being given treatment it was seen that when administering P1, P2, and P3 there was a significant difference to the control group (P0). The administration of P0 and P3 had a significant difference compared to groups P1 and P2; and P0, P1, and P2 have significant differences to P3. It can be concluded that the best treatment group for stamina is the 120 mg/kg weight (dose group (P3) which is then followed by the 60 mg/kg weight dose group (P2) and the 30 mg/kg weight dose group (P1), as well as the control group (P0) which seen from its significance ($P < 0.005$).

DISCUSSION

This study describes the effect of black cumin of different concentrations on the swimming test of mice and the possible mechanisms. Our study found that black cumin enhanced swimming or endurance capacity in mice (Figure 1). Data on the swimming survival time of the mice was calculated from the time they were put into the aquarium until the mice showed fatigue as indicated by the mouse's head sinking for 4–5 seconds (Fithria et al., 2017). Another sign of fatigue is that the mouse's body stands upright and does not move its tail again. The increase in the swimming endurance time of mice shows that there is an increase in stamina.

Black cumin seed extract (*Nigella sativa*) can increase stamina in Swiss Webster mice. The results of the Post Hoc LSD test can be explained that the best treatment group for stamina is the 120 mg/kg weight (P3) ($P < 0.005$). Giving black cumin seed extract (*Nigella sativa*) to this group of swimming mice showed an increase in stamina because it contains chemical compounds that are useful as immunomodulators and anti-inflammatories so they can improve lung function. Apart from that, the compounds contained in black cumin also function as immunomodulators by increasing the antibody titers in the body (Amunulloh & Krisdayanti, 2019).

Black cumin seed extract has been used for many years because of its pharmacological properties as an immunostimulant, anti-inflammatory, and respiratory stimulant agent (Seghatoleslam, et al., 2016). The content of black cumin seeds, one of which is the saponin contained in black cumin seed extract, has an anti-inflammatory effect and can increase the ability of skeletal muscles to oxidize free fatty acids and produce cellular energy which can support anti-fatigue activities. In addition, flavonoids can inhibit ATP from binding to calcium channels to enter the sarcoplasmic reticulum. This obstacle causes calcium to be released into the cytosol and then bind to troponin which will cause muscle contractions to last longer so that fatigue does not occur (Hesturini & Vadila, 2022).

Black cumin (*Nigella sativa*) is also understood in Islam as a cure for all kinds of diseases which is based on the hadith history, Rasulullah SAW said "Indeed in the Habbatussauda plant (black cumin) there is a cure for all kinds of diseases, without exception of death" (HR. Bukhori and Muslim). However, Ibn Hajar al-Asqalani also mentioned in the book Fathul Bari Syarah Shahuh Bukhari that Imam al-Khattabi explained that the expression "The antidote to all diseases" was used because according to him there was not a single plant that Allah SWT created in this world that did not have benefits as a treatment goal (Kurniasih et al., 2022).

CONCLUSION

In this study, a dose of black cumin extract enhanced the stamina of mice. Black cumin extract showed more effects on the survival and time reaction of mice while swimming. The possible mechanism for increased stamina capacity may be that the dose of black cumin promotes the ability of endurance in mice.

It is recommended that further research be carried out in humans. Apart from that, the data from this research can also be used as reference material for conducting further research on other mammals and clinical trial research.

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