

## The Effectiveness Test of Immunostimulants with Black Cumin Oil Extract (*Nigella sativa*) in Male Mice (*Mus musculus*)

Nurhidayanti<sup>1\*</sup>, Trimin Kartika<sup>2</sup>  
\*e-mail: nuri89\_yanti@yahoo.com

<sup>1</sup>Medical Laboratory Technology Program, Institute of Health Sciences and Technology  
Muhammadiyah Palembang, Indonesia

<sup>2</sup>Biology Program, University of PGRI Palembang, Indonesia

### ABSTRACT

The immune system is closely related to leukocytes. Leukocytes are part of the immune system which have an important role in every disease-causing agent. One of the efforts that can be made to increase the immune response is to provide an immunomodulator, a substance that can improve the immune system by means of stimulation (immunostimulants). Black cumin is able to stimulate bone marrow and immune cells and is able to protect normal cells from damage by disease agents, an increase in the total number of blood cells and their differentiation. The purpose of this study was to determine the effect of giving black cumin oil extract on leukocyte levels in male mice. This type of research is experimental, to see the levels of leukocytes, after being treated with doses of 150 mg/kgBB, 300 mg/kgBB and 600 mg/kgBB for 21 days. The data obtained were then analyzed statistically using the ANOVA test. The results obtained by the total number of leukocytes on day 7, day 14 and 21 showed a significant difference ( $p < 0.05$ ) for each treatment group. The use of black seed oil extract is very effective for boosting the immune system or immunostimulating. The benefit of this research is that it can provide information to the public that black cumin oil extract can be used as an alternative in increasing the body's immune system.

**Keywords:** immunostimulants, black cumin, male mice

### INTRODUCTION

The immune system is a system consisting of several types of cells that are fixed and attached to tissues or that are able to move (mobile) and interact in lymph networks that are spread throughout the body (Sudiono, 2014). An immune system that works well can protect the body well too. One of the efforts that can be made to increase the immune response is to provide an immunomodulator, a substance that can improve the immune system by stimulating (immunostimulating) or suppressing / normalizing abnormal

immune reactions (*immunosuppressants*) (Alkandahri et al., 2018).

The immune system (immune) deals with leukocytes. Leukocytes are part of the immune system that have an important role in every disease-causing agent. The main function of leukocytes is to destroy infectious and toxic materials through the phagocytosis process and form antibodies (Lubis et al., 2016).

The increase in the number of leukocyte cells is a response in the form of protection against the presence of foreign cells, including bacterial infections that enter the body. Leukocytes

are included in the nonspecific immune system which is the front line of defense that is ready to function when foreign objects or microbes enter the body and can provide an immediate response (Sudiono, 2014).

Immunomodulatory substances can come from natural and synthetic materials. One of the natural ingredients that have been researched and can be used as an immunomodulator is black cummin (*Nigella sativa*). Black cummin is able to stimulate bone marrow and immune cells and is able to protect normal cells from damage by disease agents, an increase in the total number of blood cells and their differentiation (Marlinda, 2015).

The content of black cummin is proven to be able to strengthen and stabilize the body's immune system by increasing the ratio between T-helper and T-suppressor cells by 55% with an average attainment of natural killer cell activity by 30%. Black cummin is able to stimulate bone marrow and immune cells, protect normal cells from cell damage by viruses, destroy tumor cells and increase the number of antibodies produced by B-cells (Akrom et al., 2015). Based on the above studies, the researcher intends to test the effectiveness of the Immunostimulant Extract of Black Cummin Oil (*Nigella sativa*) in male mice (*Mus musculus*).

The purpose of this study was to determine the effect of giving black cummin oil extract (*Nigella sativa*) on leukocyte levels in male mice (*Mus musculus*). The benefits of this research are that it can provide information to the public that black cummin oil extract can be used as an alternative in increasing the body's immune system.

## MATERIAL AND METHOD

This type of research is experimental. This research was

conducted in September-October 2020 at the Hematology Laboratory of the Muhammadiyah Palembang Institute of Health and Technology. The subjects of this study were male mice aged 6-8 weeks with a body weight of 20-23 grams. Given food in the form of granules (pellets).

This research was started by acclimating the test animals for 2 weeks, each group consisting of 5 mice. The total number of mice used was 20 mice divided into 4 treatment groups based on the dose of black cummin oil extract given. The cummin oil extract was administered orally at a dose of 150 mg / kg, 300 mg / kg, and 600 mg / kg and one control group. Every 7th, 14th day, and 21st day blood was drawn through the eye retro orbital plexus of mice. The blood sample for the total leukocyte test was inserted into the EDTA vacutainer tube.

The total leukocyte count was calculated using a hemocytometer with a 1:20 dilution. To obtain a 1:20 dilution, the blood sample was homogenized, then sucked using a leukocyte pipette and aspirator until it was 0.5 degrees. Next, the Turk solution is sucked up to 11 tera, the aspirator is removed then homogenized manually, by rotating it to form a figure 8. Then the sample is discarded about 2-3 drops, after that it is put into a Neubauer counting room and covered with a cover glass then examined under a microscope magnification 40 x 10. Leukocytes are counted in four large squares in each corner of each side of the counting chamber. The cells attached to the dividing line on the left and on the top line of the rectangular box are counted, cells that are attached to the other two sides of the box are not counted (Muslimah, 2016).

Analysis of the total number of leukocytes using ANOVA (Analysis of Variance) using the SPSS 17.0 program for windows, the confidence level is 95% with ( $\alpha = 0.05$ ).

## RESULTS AND DISCUSSION

The results of the calculation of the total number of leukocytes on day 7, day 14 and day 21 in male mice that were given black cumin oil extract at low doses (150 mg / kgBB), medium doses (300 mg / kgBB) and high doses (600 mg / kgBB) were obtained. The following results:

Table 1. Results of The Total Number of Leukocytes (per mm<sup>3</sup>)

Group of Mice	Average of Leukocytes Number		
	Day 7	Day 14	Day 21
Control	4,5 ± 1,0	4,8 ± 1,3	5,3 ± 1,5
Low Dose	6,5 ± 1,5	8,5 ± 1,8	9,5 ± 2,3
Medium Dose	7,9 ± 1,1	11,0 ± 1,5	11,6 ± 2,2
High Dose	11,3 ± 2,5	11,6 ± 3,5	12,0 ± 3,5

Table 1. Above shows the total number of leukocytes in the control group days 7, 14 and 21 are in the normal range. Normal range: the total number of leukocytes in male mice is 4 - 12 x 10<sup>3</sup> per mm<sup>3</sup> (Tree et al., 2014). The total number of leukocytes on day 7 of the low, medium and high dose black cumin oil extract group was higher than the control group. The total number of leukocytes increased as the dose of black cumin oil extract was given. The group of high doses of black cumin oil extract had the highest total number of leukocytes.

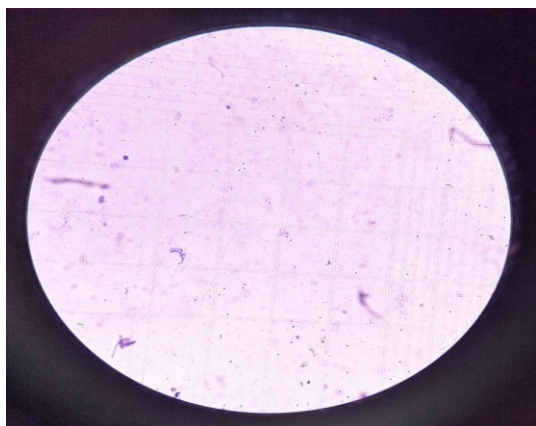


Figure 1. The result of the microscopic examination of leukocytes

Figure 1 above shows the results of the number of leukocytes observed through a microscope with a magnification of 40 x 10.

The Anova test results on the leukocyte count on day 7 showed a significant difference (p <0.05). From these results, it is explained that the addition of cumin oil extract can increase the number of leukocytes in mice.

Black seed oil extract works as an immunostimulant and increases the production of antibodies in the body of mice. The mechanism of action as an immunostimulant is through a non-specific immune system, namely by increasing the activity and number of white blood cells (leukocytes) and through the specific immune system, especially in the specific cellular immune system by increasing the ratio between T helper cells and T suppressor cells (Dontriska, 2014).

Anova test results on day 14 showed a significant difference in the number of leukocytes in each group (p = 0.020). The results of this study are in line with Dontriska's (2014) study which showed that the treatment with added black cumin was higher than the number of controls that did not add black cumin.

This research is also in line with the research of (Aldi & Suhatri, 2015) where the administration of ethanol extract of black cumin at a dose level of 50 mg / kgbb, 100 mg / kgbb and 200 mg / kgbb in mice given goat erythrocyte suspension antigen 5% can increase the number of leukocytes, lymphocytes, and significant monocytes.

Anova test results showed that there was a significant difference between the control group and the moderate dose group (p = 0.008), the control group with the high dose group (p = 0.008) and the moderate group with the high dose group (p = 0.008).

Anova test results on day 21 showed a significant difference in the number of leukocytes in each group ( $p = 0.017$ ). This research is in line with research of (Zikriah, 2014) which shows that giving ethanol extract of black cumin at doses of 125 mg / kgbb, 250 mg / kgbb and 500 mg / kgbb can increase the total number of leukocytes and the percentage of lymphocytes in BABL / C mice. According to (Akrom et al., 2015) that the Thymoquinone compound in black cumin is a compound that can boost the immune system and as an immunostimulant. Giving black cumin can increase the antibody titer which is a specific immune system and increase the number of leukocytes which are non-specific immune systems.

Comparison of total leukocytes between days 7, 14 and 21 using anova test showed a significant difference between days 7, 14 and 21  $p = 0.00$ . The results of the study of data on the total number of leukocytes that are at the normal high limit according to (Yenita, 2017) the total number of leukocytes that are at the highest limit of normal shows that the immune system produces a sufficient total number of leukocytes in the blood circulation to fight infection. An increase in the total number of leukocytes indicates the ability of the immune system to fight infection or foreign bodies. Leukocytes, which are the natural (specific) immune system, play an important role in protecting the body from attack by microorganisms.

## CONCLUSION

Based on the results obtained, it can be concluded as follows, the administration of black cumin (*Nigella sativa*) oil extract at doses of 150 mg/kg, 300 mg/kg and 600 mg/kg had an effect on the number of leukocytes in mice (*Mus musculus*). There is a difference in the ratio of total leukocytes between days

7, 14 and 21 using the ANOVA test showing a significant difference between days 7, 14 and 21  $p = 0.00$ . The total number of leukocytes increased with increasing dose of black cumin oil extract given. The high-dose black cumin oil extract group had the highest total number of leukocytes.

## REFERENCES

- Akrom, Widjaya, A., & Armansyah, T. (2015). Ethanol Extract of Black Cumin (*Nigella sativa*) Seed Increases Macrophage Phagocytic Activity of Swiss Mice Infected with *Lysteria monocytogenes*. *Jurnal Kedokteran Hewan*, 9(2), 94–100.
- Aldi, Y., & Suhatri, S. (2015). Aktivitas Ekstrak Etanol Biji Jintan Hitam (*Nigella sativa* Linn.) terhadap Titer Antibodi dan Jumlah Sel Leukosit pada Mencit Putih Jantan. *Scientia : Jurnal Farmasi Dan Kesehatan*, 1(1), 35. <https://doi.org/10.36434/scientia.v1i1.14>
- Alkandahri, M. Y., Subarnas, A., & Berbudi, A. (2018). Aktivitas Immunomodulator Tanaman Sambiloto (*Andrographis paniculata* Nees). *Farmaka*, 16(3), 16–20.
- Dontriska. (2014). Efektivitas tepung jintan hitam (*Nigella sativa*) untuk mencegah infeksi *Aeromonas hydrophila* pada ikan patin. *Jurnal Akuakultur Rawa Indonesia*, 2(2), 188–201.
- Lubis, N. G., Sugito, S., Zuhrawati, Z., Zuraidawati, Z., Asmilia, N., Hamny, H., & Balqis, U. (2016). Efek Peningkatan Suhu Terhadap Jumlah Leukosit Ikan Nila (*Oreochromis niloticus*). *Jurnal Medika Veterinaria*, 10(1), 31. <https://doi.org/10.21157/j.med.vet.v10i1.4033>
- Marlinda, L. (2015). Efektivitas Ekstrak Etanol Biji Jintan Hitam (*Nigella*

- sativa* Linn.) Terhadap Peningkatan Fagositosis dalam Respon Imun Tubuh. *Journal of Majority*, 4, 58–64.
- Muslimah. (2016). Perbedaan jumlah trombosit pada 25, 12,5 dan 5 kotak sedang bilik hitung improved neubauer skripsi. *Progam Studi D IV Analisis Kesehatan Fakultas Ilmu Keperawatan Dan Kesehatan Universitas Muhammadiyah Semarang*, 1–37.
- Sudiono, J. (2014). Sistem Kekebalan Tubuh. *Penerbit Buku Kedokteran EGC, June*, 1–86.  
<http://www.ncbi.nlm.nih.gov/pubmed/810049>  
<http://doi.wiley.com/10.1002/anie.197505391>  
<http://www.sciencedirect.com/science/article/pii/B9780857090409500205>  
<http://www.ncbi.nlm.nih.gov/pubmed/21918515>  
<http://www.cabi.org/cabebooks/ebook/20083217094>
- Tree, W., Extract, B., Fahrimal, Y., Rafina, A., Azhar, A., & Asmilia, N. (2014). Profil Darah Tikus (*Rattus norvegicus*) Yang Diinfeksi *Trypanosoma evansi* Dan Diberikan Ekstrak Kulit Batang Jaloh (*Salix tetrasperma* Roxb). *Jurnal Kedokteran Hewan - Indonesian Journal of Veterinary Sciences*, 8(2).  
<https://doi.org/10.21157/j.ked.hewan.v8i2.2653>
- Yenita, Y. (2017). Uji Efektivitas Pemberian Minyak Jintan Hitam (*Nigella sativa* L.) terhadap Kadar Gula Darah Mencit Diabetes Melitus yang Diberi Aloksan. 2(2), 101–115.
- Zikriah. (2014). Uji imunomodulator ekstrak etanol jinten hitam (*Nigella sativa* L.) Terhadap Jumlah Total Leukosit, Persentase Limfosit, Persentase Monosit dan Kadar Interleukin-1 $\beta$  Pada Mencit BALB/c. *UIN Syarif Hidayatullah Jakarta*.