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by Erma Safitri

Submission date: 12-Feb-2020 04:00PM (UTC+0800)

Submission ID: 1256034875

File name: Bukti_C34_Immunomodulatory_Activity_of....pdf (88.03K)

Word count: 1620

Character count: 8385

Immunomodulatory Activity of Black Jinten Oil (*Nigella sativa*) as Macrophage Activator for *Salmonella typhimurium* Infected Rat

Dewa K. Meles, Erma Safitri¹, Wurlina, Imam Mustofa, Suherni Susilowati and Desak K.S.C. Putri

Veterinary Medicine Faculty, Universitas Airlangga, Surabaya 60115, Indonesia.

(Received : July, 2019 249/19 Accepted : September, 2019)

Abstract

This study was aimed to prove immunomodulatory activity of *Nigella sativa* as macrophage activator for *S.typhimurium* infected rats. 125 rats were divided into five groups and observed for five days (2nd, 4th, 6th, 8th, 10th). The rats were infected with *S.typhimurium* 10⁵CFU by intraperitoneal route, and given black jinten oil doses 12.60 mg, 18.90 mg, 25.20mg/200 gram bw/day, for 10 days. The observation: total number of leukocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes, activated macrophages. The results showed that black jinten oil was able to maintain an immune response towards normal total number of leukocytes and activated macrophages, in rats which were infected with *S.typhimurium*.

Key words : Black jinten oil, Immunomodulators, Salmonellosis

Nigella sativa is natural immunomodulator and often used to strengthen the immune system from attacks by viruses, germs, bacteria and extreme changes in conditions. Jinten seed oil contains several chemicals that have activities as hypo-allergenic, antihistamines anti-inflammatory and immunodeficiency (Akpolat and Kanter, 2009). So that it can be used as a medicine to prevent and treat for immune deficiency, such as in Salmonellosis case.

Materials and Methods

125 rats were divided into 5 groups and observed for 5 days on (2nd, 4th, 6th, 8th, 10th). The treatment group was infected with *Salmonella typhimurium* at 10⁵ CFU intraperitoneally (Chart, 2003,

Mustofa *et al.*, 2017). The treatment groups were given black jinten oil at doses of 12.60 mg (T1), 18.90 mg (T2) and 25.20 mg (T3) per 200 gram bw/day for 10 days and compared with control negative (C-) = healthy rats and control positive (C+) = rats with salmonellosis and without black jinten oil. The parameters observed were total number leukocytes, neutrophils, eosinophils, basophils, lymphocyte, monocytes and activated macrophages. The data was analyzed by one-way analysis and, if there were differences, further analysis was carried out with the smallest real difference test

Results and Discussion

The results of the examination of total leukocytes (neutrophils, eosinophils, basophils, lymphocytes, monocytes) and activated macrophages in rats infected with *Salmonella typhimurium*, then treated with black jinten oil extract at various doses, are presented in Table I and II.

The results of the study showed that administration of black jinten oil extract for 2 days, 4 days, 6 days, 8 days and 10 days in rats infected with *Salmonella typhimurium* between the control negative group (C-) and control positive group (C+) and T1, significant differences ($p < 0.05$), while T2 and T3 there is no difference ($p > 0.05$) with C-. The results revealed that in rats infected with *Salmonella typhimurium* had an increase in total leukocytes neutrophils, eosinophils, basophils, lymphocytes, monocytes and activated macrophages from infection day 2, to 4, to 6, to 8 and day 10 in C+ and T1 compared to the group of normal rats (C-).

Circumstances that can stimulate an increase in leukocyte levels (leukocytosis)

¹Corresponding author : Email : rma_fispro@yahoo.com

Table I. Average of total leukocytes in rats administered with black jinten oil extract after infected with *Salmonella typhimurium* (Mean \pm S.E.)

Treatment	Average of Total Leukocyte Count in Days				
	2nd	4th	6th	8th	10th
C-	7880 ^a \pm 228.03	7900 ^a \pm 158.11	7960 ^a \pm 207.36	7860 ^a \pm 230.21	7920 ^a \pm 389.87
C+	13940 ^b \pm 194.93	13820 ^b \pm 192.35	13900 ^b \pm 187.08	13960 ^b \pm 89.44	13920 ^b \pm 130.384
T1	10780 ^c \pm 861.39	10540 ^c \pm 746.99	10680 ^c \pm 589.06	10220 ^c \pm 438.17	10140 ^c \pm 804.98
T2	7980 ^a \pm 526.30	8040 ^a \pm 89.44	8020 ^a \pm 715.54	7880 ^a \pm 356.37	7940 ^a \pm 642.65
T3	7960 ^a \pm 114.01	7940 ^a \pm 650.38	7940 ^a \pm 384.70	7800 ^a \pm 187.08	7780 ^a \pm 228.03

Different letter superscripts in one column indicate differences ($p < 0.05$)

indicate a change in the normal body reaction to the entry of foreign material into the body including stress and infection by germs and viruses (Davis *et al.*, 2008).

Administration of black jinten oil extract at a dose of 18.90 mg / head / day in rats infected with *Salmonella* were able to stabilize the normal body condition by maintaining the total leukocytes. The nonspecific immune response in the body can be maintained on the administration of black jinten oil fraction after infection with *Salmonella typhimurium*, indicates that black jinten oil extract can modulate the total leukocytes in the body towards normal and reduce the burden of leukocytes, during infections.

Leukocyte programmed death was mediated by the release of signaling molecules called ligands. Furthermore, the ligand will bind with the receptor found in leukocyte transmembrane. Death receptors located on the cell surface are related to the Tumour Necrosis Factor (TNFR) receptors that initiate leukocyte death by apoptosis.

Administration of black jinten oil extract starting at a dose of 18.90 mg / head / day in rats infected with *Salmonella typhimurium* was able to stabilize the body condition by maintaining the amount of Eosinophils, Basophils, Neutrophils, Lymphosit, Monosit and activated Macrophages within the normal range. Black jinten extract is able to modulate the amount of Eosinophils in the body towards normal and replace the role and function of Eosinophils at the time of infection.

The number of lymphocytes shows the body immune response related to T cells and B cells in maintaining the body resistance if an infection occurs. T cells will differentiate into cytotoxic T cells, helper T cells, suppress T cells, and memory T cells that play a role in the secretion of Interferon IF (IFN γ) in activating macrophages to phagocyte infectious microorganisms that enter the body. While B cell will differentiate into memory cells and plasma cells which produces antibodies (Cummings, 2005; Mustofa *et al.*, *loc cit*). The immunomodulatory response due to black jinten oil extract is related to the increase in differentiation and proliferation activity of T cells and B cells.

Decreased macrophages towards normal numbers shows the immunological response of rats to *Salmonella typhimurium* and treated with black jinten oil extract by the body resistance through increased neutrophils, basophils and eosinophils (Criss *et al.*, 2001) while decreasing the number of macrophages.

The administration of black jinten oil extract after *Salmonella typhimurium* infection did not cause a significant increase in the number of macrophages compared to normal rats. Activated macrophages play a role in phagocytic process. The role of macrophages and activation of macrophages depends on gamma interferon produced by T cells, B cells, NK cells and activated macrophage cells (Kusmardi *et al.*, 2007, Mustofa *et al.*, *loc cit*). The black jinten oil extract has been able to stabilize and support the role of macrophages in maintaining immune responses.

Table II. Average of Macrophage count on rats administrated with black jinten oil extract after being infected with *Salmonella typhimurium* (Mean \pm S.E.)

Treatment	Average of Rats Macrophage Count in Days				
	Ke 2	Ke 4	Ke 6	Ke 8	Ke 10
C-	13.20 ^a \pm 2.16	13.40 ^a \pm 2.07	12.80 ^a \pm 1.30	13.40 ^a \pm 1.94	12.80 ^a \pm 1.30
C+	38.80 ^b \pm 9.57	38.20 ^b \pm 1.78	37.40 ^b \pm 2.30	37.40 ^b \pm 3.36	37.80 ^b \pm 3.11
T1	26.80 ^c \pm 5.40	27.40 ^c \pm 2.34	27.20 ^c \pm 1.92	27.60 ^c \pm 4.39	26.80 ^c \pm 4.32
T2	14.20 ^a \pm 1.30	14.80 ^a \pm 1.09	14.40 ^a \pm 2.30	14.20 ^a \pm 1.30	13.80 ^a \pm 1.78
T3	12.80 ^a \pm 2.38	12.60 ^a \pm 0.54	12.60 ^a \pm 2.79	12.40 ^a \pm 2.30	12.20 ^a \pm 2.28

Different letter superscripts in one column indicate differences ($p < 0.05$)

Administration of black jinten oil extract with dose of 18.90 mg / head / day in rats infected with *Salmonella typhimurium* from day 2 to day 10 has been able to stabilize the body immune response by maintaining the number of macrophages towards normal.

Summary

The Black jinten oil extract can stabilize total leukocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes and activated macrophages in rats to normal levels which were infected with *Salmonella typhimurium*

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