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The Potential Of Black Cumin Extract (*Nigella Sativa L.*) On lymphocyte Proliferation Wistar rat Induced With Hepatitis B Vaccine.

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ABSTRACT

The aim of the research was to examine the potential of Black Cumin (*Nigella sativa L.*) extract on lymphocyte proliferation wistar rat induced with hepatitis B vaccine. The research sample was male wistar rat (*Rattus norvegicus*) divided into negative control group, positive control group (induced by hepatitis B vaccine), treatment group 1 (Hepatitis B vaccine and black cumin extract dose 75 mg / kgBB) and group 2 (induced Hepatitis B vaccine and black cumin extract dose 150 mg / kgBW). This research performed liver histology examination with staining of Hematoxylin Eosin (HE). Based on the result, it can be found the negative control group has the average number of lymphocyte cells 114.5 cells / plp, whereas the positive control group has the average number of lymphocytes cell 143.1 cells / plp. The average of leukocyte cells in treatment group 1 is 130.8 cells / plp, and the mean of leukocyte cell in treatment group 2 is 154.7 cells/plp. Based on the statistical Analysis, there is a difference between the number of lymphocyte proliferation cells in treatment 1 with treatment 2 ($p = 0,002$) and the optimal dose of black cumin is 150 mg / KgBB.

Keywords: Black Cumin (*Nigella sativa L.*), lymphocyte proliferation, Hepatitis B.

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INTRODUCTION

Basic health research of Ministry of Health in 2017, the 7.1% Indonesian population has been gotten hepatitis B disease. A few areas in Indonesia have prevalence rates of hepatitis B namely East Nusa Tenggara, Papua, South Sulawesi, Central Sulawesi, Maluku, Southeast Sulawesi, North Sulawesi, NAD, Central Kalimantan, South Kalimantan and North Sumatra.[1] Hepatitis B virus may occur on the surface of the environment even though no blood is present and can still cause transmission disease. If the infection has persisted, it can be led to liver damage or liver cirrhosis. [2,3] Hepatitis B virus will do replication in hepatic cells in 3 days after being in the patient's blood circulation.[4] This can bring to inflammation of the liver and trigger the body's immune response to hepatitis B infection.[4,5] Hepatitis B virus enters the body and is recognized by receptors, then presented APC (Antigen Presenting Cell) by MHC (Major Histocompatibility). Activation of lymphocytes from triggered antigen through APC will lead to lymphocyte proliferation. Lymphocyte cell proliferation is affected by interleukin-2 (IL-2) to proliferate into Th1 and Th2. Increased proliferation is used as an indicator of increased immune system activity against infection.[6,7,8]. In the time of treatment and prevention efforts undertaken by the community to overcome the hepatitis B virus infection is limited to the chemical drug and vaccination use. Treatment by using chemicals also have not given satisfactory results. Prevention with vaccines does provide hope, but its impact on the new community will be visible after decades.[9] Some options for hepatitis B treatment have been used such as lamivudine, entecavir and peg-interferon, but treatment success is limited to only about 65-70%. The alternative prevention can be used to hepatitis B infection is with the utilization of herb. One of the herb can be used is black cumin (*Nigella sativa* L.) Black cumin (*Nigella sativa* L.) has several ingredients such as amino acids, proteins, alkaloids, saponins, tannins, nigelin (bitter substances), nigellone and essential oils.[9,10] One of the ingredients as anti-inflammatory and an immunomodulator in black cumin is thymoquinone.[11]

Compounds that have immunostimulator by activating T lymphocytes, NK cells, and macrophage cells. the body's resistance to microbial infection depends on the activation of lymphocyte cells T cell lymphocytes will be activated, differentiated and proliferate when triggered by antigen or mitogen. The ability of these cells to proliferate or form clones demonstrates the ability of immunologic responses.[12] According to the research, thymoquinone compounds are a major component of essential cumin essential oils that have immune boosting capabilities and as immunostimulants. [13] Thymoquinone has also shown to increase the number of neutrophils by stimulating Toll-like Receptor (TLR) expression in neutrophils thereby increasing the activity of phagocytosis. This activation of TLR increases the activity of phagocytic cells such as PMN neutrophils so that the number of PMN neutrophils will increase.[13, 14]

A research of the immunomodulator effects of black cumin shows the administration of black cumin ethanol extract with a dose of 250 mg / kgBW could increase the total number of leukocytes and lymphocyte percentage in BALB / C mice.[15] Accordingly, there is a need for research on the utilization of natural materials such as black cumin as an alternative pattern of therapy against hepatitis B virus infection, thus reducing the side effects of the use of chemicals for the treatment and prevention. The aim of this research is to know Potential of Black Cumin Extract (*Nigella sativa* L) on Wistar-induced Lymphocyte rat Rupture Proliferation Hepatitis B Vaccine so black cumin (*Nigella sativa* L.) which can be used as a natural immunomodulator in a preventive effort and as a treatment for Hepatitis B.

METHOD

The type of research is laboratory experiment with posttest only control group design design. The samples used in this research were male Wistar mice (*Rattus norvegicus*). The process of black cumin extraction was done by maceration method using 96% ethanol solvent. The solution is macerated for 24 hours at room temperature. Filtering and concentrating with a rotary vacuum evaporator at temperature 50 will be obtained the extract with 100% concentration. Black cumin extract is made dose 75 mg / kgBB and 150 mg / kgBB. In this research, twenty-four rat were divided into four treatment groups. Two control groups were negative control group which were not given black cumin extract nor induced hepatitis B vaccine and positive control group only induced by hepatitis B vaccine.

Treatment group 1 daily was given black cumin extract for 7 days with dose 75 mg / kgBB and treatment group 2 every day given black cumin extract for 7 days with dose 150 mg / kgBB as much as 1.8 mL once daily, then induced by hepatitis B vaccine in intraperitoneally on the 7th day of treatment. On day 8 On

all mice were dyuthanized and dincropsed. Liver is taken and examined for further processing in the preparation of histopathologic preparations. Histopathologic examination process performed liver cut and fixed in 10% NBF for 24 hours. after streaming, and put in a tissue cassette. Then put into tissue processor for dehydration stage, clearing, embedding. and blocking was done with paraffin. Subsequently the preparation was stained with hematoxylin Eosin (HE).

RESULT AND DISSCUSSION

Based on the results of 24 Wistar rat which were grouped into 4 groups: negative control group, positive control, treatment 1 and treatment 2 were all conditioned by hepatitis B except negative control group. Further examination of lymphocyte cells that experience proliferation of the liver for histopathologically processed. This research was carried out for 8 days in male wistar rat selected because according to pharmacological test ie male rat can reduce physiological variations especially the female hormone cycle during the estrogen cycle that can affect the immune response of rat.

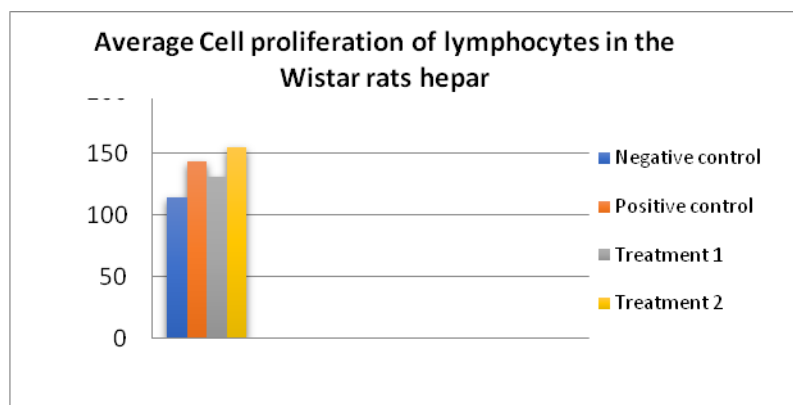
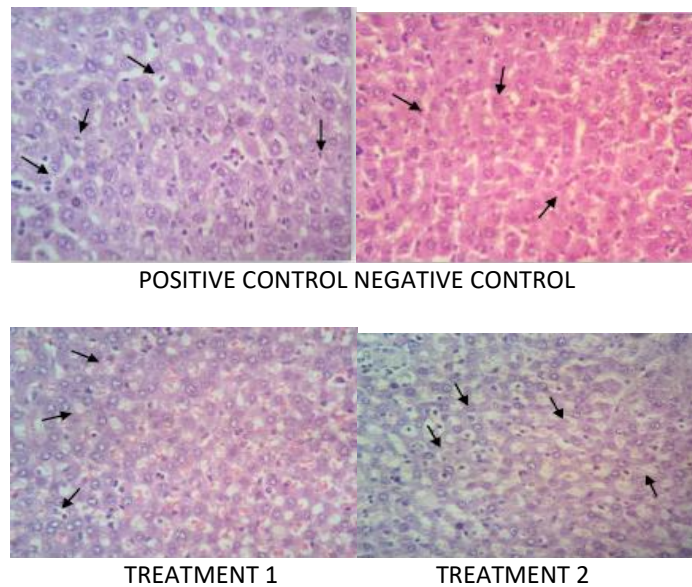


Figure 1: Average of lymphocyte cell proliferation in wistar rats liver

After giving black cumin extract dose 75 mg / KgBB and induced with hepatitis B vaccine, the average number of lymphocyte cells in rat increase compared with negative control and the value of positive control. The number of lymphocyte cell proliferation in the positive control group is higher than in the negative control group. The increased number of lymphocyte proliferation cells in this positive control group is a sign of inflammation. In this positive control group is indicated that the possibility of an inflammatory process caused by HBsAg antigen surface contained in hepatitis B vaccine induced in rat.

The lymphocyte proliferation cell in treatment 1 compared with the positive control group has not increased, but the number of proliferation cells increases compared with the control group.

In Post Hoc test is not different or not significant between treatment group 1 with positive control ($p = 0,076$) $> \alpha 0,05$. This is due to extract of black cumin dose 75 mg / KgBB shows still inflammation and thymoquinone content in black cumin extract is able to activate lymphocyte cells in the liver experience of proliferation so that the number increases but not yet able to eliminate the incoming virus pathogens. This indicates that the Hepatitis B (HBsAg) Antigen surface in the induced hepatitis B vaccine can stimulate the inflammation in the mice body. This infamation process is characterized by an increase in the number of lymphocyte cells in the liver.¹⁶ HBsAg is a specific protein surface of hepatitis B virus expressed on the surface of the hepatocyte and via presenting cell antigen (APC) presented to T helper lymphocyte cells. Activated T helper lymphocyte cells trigger the formation of T lymphocyte proliferation.¹⁷

Treatment group of 2 wistar mice induced by hepatitis B vaccine, then given black cumin extract dose 150 mg / KgBB orally known amount of lymphocyte proliferation increase compared to comparison with control group. The result of Post Hoc test shows ($p = 0,000$) $< \alpha 0,05$ which means there is difference between treatment group 2 and negative group. An increase in the number of lymphocyte cells in the treatment group of 2 wistar mice treated with black cumin extract dose 150 mg / KgBB compared to all control groups, proving that the content of thymoquinone in black cumin extract at this dose can overcome the infectious virus infections so that no inflammation occurs.

This was demonstrated by lymphocyte cells that experienced proliferation of 154.7 cells / plp and wistar mice did not experience inflammation. The mechanism of thymoquinone as anti-inflammatory acts as a cyclooxygenase pathway and lipooksigenase blockage of arachidonic acid metabolism.^{18,19} The presence of obstacles in the cyclooxygenase pathway which affects the decrease of PGE2 and proinflammatory cytokines (IL-1, IL-2, IL-6 and TNF- α) will affect the decrease in phagocytosis activity. Barrier of lipooksigenase pathway that influences leukotrien production known as mediator of leukocyte activity.

The Post Hoc test on treatment 1 and treatment 2 has a significant difference ($p = 0.002$) $> \alpha 0.05$, which showed the dose of black cumin extract 75 mg / KgBB was able to activate lymphocyte cell proliferation but the dose of black cumin extract 150 mg / KgBB more activate lymphocyte cells to proliferate in the liver. According to Marlinda (2015), extract of black cumin ethanol with doses of 50 mg / kgBW, 100 mg / kgBW and 200 mg / kgBW may increase the percentage increase of lymphocyte cells in white mice lymph.¹⁷ This is due to the linkage of thymoquinone content in black cumin extract at dose. The greater dose of black cumin extract has the greater potential to increase the proliferation and differentiation of lymphocyte cells. The Tyastuti's research (2006) says the more active number of T lymphocytes the greater the number of tumor growth inhibitor cells (TNF- α , IFN- γ , and IL-2R) expressed so that tumor cells are more easily suppressed its development. Tumor Necrosis Factor- α (TNF- α) is produced by active T lymphocytes and together with Interferon- γ (IFN- γ) is cytotoxic to the Hepatitis B viral antigen. IFN- γ plays a significant role in defense against viral attack because it increases class I and II MHC expression that play a role in the introduction of antigen produced by Hepatitis B virus. ^{19,20}

Black cumin extract of thymoquinone has an immunomodulatory effect that stimulates stimulated T lymphocytes to produce interferon- γ (IFN- γ) and interlukin-2 (IL-2) cytokines. IFN- γ will play a role in the activation of macrophage cells and may induce the expression of a major class II histocompatibility complex (MHC) molecule on macrophage cells, thus helping macrophage cell function in lymphoid follicles to recognize foreign substances. Macrophage cells can also release cytokines, IL-1, which play a role in stimulating the proliferation of Th cells. IL-2 not only plays a role in the expansion of T cell clones after known antigen, but it also increases the proliferation and differentiation of other immune cells.¹² The effects of black cumin extract can stimulate Macrophage Activating Factor (MAF) cytokines, thereby enhancing the function of macrophages that play a role in the cellular immune system. Thymoquinone is able to increase the function of immune cells both cellular and humoral.²¹ The macrophage cells have an important role in the immune system as non-specific defense early against the invasion of microorganisms as well as antitumor immunity with its function as as professional phagocytes to destroy the antigen and present to the lymphocyte cells. The macrophage cells activated will execute the effect or function as activators of cell lymphocytes, mikrobisidal and tumorisidal. ¹² Treatment group 1 and group 2 known treatment there was no damage to the liver cells or hepatocytes. It is marked on the slides histopathology on the treatment group 1 and treatment group 2 there is no obstructive

or tissue necrosis liver. Likely due to cell proliferation of lymphocytes which the presence of black cumin Thymoquinone days materials capable of keeping antigen Hepatitis B virus attacks so that forms, core and chromatin hepatocyte cells normal. Cumin 1 gram/kg in histopathology is able to protect the liver of tissue damage. 23,24

CONCLUSION

Based on the results of research and discussion on the potential of black cumin extract (*Nigella sativa* L.) on the proliferation of wistar rat (*Rattus norvegicus*) induced by hepatitis B vaccine, it can be concluded that the difference between the number of lymphocyte proliferation cells in treatment 1 with treatment 2 ($p = 0.002$), and the optimal dose of black cumin is 150 mg / KgBB.

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