# Antiimmunotoxic of Black Cumin Seed Oil (*Nigella sativa* Oil) in DMBA (Dimethylbenzantracene)-Induced Mice

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***Abstract*—A single and repeated doses of DMBA are toxic to immune system. Black cumin seed oil (BCSO) is immunoprotector agent. A study was performed to identify antiimmunitoxicity effects of BCSO on DMBA-induced SD mice. This study was conducted with an experimental design with BCSO administration group and a normal control group. DMBA induction was administered by two methods: a single dose of 15mg/mouse per oral (DMBA15group), and repeated doses of 10 × 20mg/kgb twice/week for 5 weeks (DMBA20/group). Normal control was a non-DMBA-induced group while diseased groups were DMBA-induced groups, DMBA15 and DMBA20. Treatment groups were DMBA 15 and DMBA 20 which were given 0.25ml/kgBCSO 14 days before and during DMBA induction (DMBA15BCSO and DMBA20BCSO groups. Observation was carried out on peripheral blood, spleen histological description and spleen weight, CD4 and CD4CD25 lymphocyte counts. Results showed that the single and repeated doses of DMBA in SD mice are immunosuppressive and hematotoxic. The administration of 0.25ml/kgbwBCSO can decrease the immunosuppressive and hematotoxic effects of DMBA. The number of leukocyte and CD4Th in the treatment groups was higher than the diseased group ($p$ < 0.05). Conclusion: BCSO is the antihematotoxic immunoprotector for diseased groups.**

***Index Terms*—DMBA, black cumin seed oil (BCSO), immunotoxic, hematotoxic**

## I. Introduction

Dimethylbenz(a)anthracene (DMBA) is one of polycyclic aromatic hydrocarbon (PAHs) that is carcinogenic immunosuppressive. It has been proven that the active metabolite of DMBA, i.e. 3.4, dihydrodiol, 1.2, peroxides are hematotoxic and immunosuppressive, namely, to suppress the erythropoiesis process of bone marrow and to decrease lymphocyte proliferation activity in spleen tissue. It is suspected that repeated exposures of DMBA produce more carcinogenesis and immunotoxic effects than a single exposure of DMBA [1]-[3].

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Leucocytes and blood cellular components serve as the main defender against pathogens entering the body. Monocytes and neutrophils are professional phagocytes in the blood that destroy any pathogens interfering the blood. Neutrophils and other granulocyte cells also play a role in inflammatory reactions. The components of blood lymphocytes are responsible in generating adaptive immune response [1], [2], [4].

The Treg lymphocytes of CD4 and CD4CD25 play an important role in cellular adaptive immune response. The Th cells of CD4 play a major role in facilitating the adaptive immune response while the Treg CD4CD25 serve as prevention against allergic reaction and the onset of autoimmunity [4].

The main content of black cumin seed oil involves evaporating such thymoquinone, nigellone and nigelline and non-evaporating oil such as unsaturated fatty oils, i.e. linoleic and linolenic. Unsaturated fatty acids and thymoquinone are powerful antioxidants and potential for immunomodulatory and chemo preventive agents in mammary cancer). The application of N. sativa ethanol extract for 14 days in DMBA (7, 12-di-methylbenz (a) anthracene)- induced mice is able to increase TNF-α level and DNA fragmentation [5]-[9]. It has been reported that N sativa oil (crude oil fixed) and its active compound, i.e. thymoquinone, inhibit cyclooxygenase pathway and 5-lipooxygenase of arachidonic metabolism in the peritoneal cavity leukocytes of mice. However, no anti immunohematotoxic activity assay of BCSO has been conducted in DMBA-induced SD mice with repeated doses. Data availability on the mechanism of BCSO herbal immunomodulatory action is highly important for further development of this natural material [10]-[13].

## II. Research Method

Experimental design was adopted in this research with a control group of DMBA induced female SD mice. Instruments and materials exploited in this research involved corn oil, dimethylbenzantracene (DMBA), SD mice, cages, minor sets, mice feeding, handscoon,


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chloroform and other consumables; examination kits for lymphocytes of CD4 and CD4CD25 involved FITC CD4 antibody, PE CD25, PBS, RPMI; HE staining kit.

Working procedures:

(1) Examination was conducted on peripheral blood as the parameters of antiimmunotoxicological test on BCSO. Quarantine and acclimation of tested animals were carried out. Sixty SD strain mice aged two weeks were put in plastic cages covered with woven wire. The mice were treated under similar conditions and feeding. Before being exploited for the research, one week adaptation was provided for the mice in the rearing cage. During the quarantine and acclimation periods, feeding and drinking were given to the mice according to the standard. On the sixth day of acclimatization, the tested animals were randomly divided into five groups.

Twenty-five male SD strain mice aged three weeks were randomly divided into five groups. Group I as normal group was given with standard feeding and drinking during the test. Group II and group III served as diseased groups. Group II were orally given with a single dose of DMBA in corn oil at 15 mg/mouse Group III were provided with DMBA in corn oil at a dose of 10 x 20 mg / kg orally given, twice a week. Groups IV and V served as treatment groups, given with a pretreatment dose of BCSO 0.25 ml/kgbw for 14 days and induced with 15 mg DMBA/ mouse or 10x20mg/kgbw DMBA, 2 times/week per oral. The examination on peripheral blood, the count and types of leukocytes and erythrocytes was done with spectrophotometers hematoanalyzer as the previous procedure.

(2) Histopathological and spleen weight examination [2], [3], [14].

Isolation and spleen weight examination were carried out based on the procedures specified by the institution. Spleen was taken from the abdominal cavity as soon as peritoneum was opened, after anesthesia with chloroform was conducted on the mice. Following alcohol disinfectant was applied, the abdominal wall was opened with a knife and tweezers to open the peritoneum bag. After the peritoneum was opened and the abdominal content was visible, by using tweezers, the abdominal organs covering the spleen were removed and the base of the spleen was cut. After the spleen was lifted, then it was cleaned from connective tissues. After totally being clean, then spleen was weighed by using calibrated digital scales. Following the spleen was weighed, the it was cut for histopathological examination and the isolation of spleenocytes.

The histopathological examination of the spleen was performed based on the procedures of the previous researchers, from processing the tissues, hematoxylin & eosin staining and histopathological analysis. The H & E preparation was descriptively analyzed on the changes occurred at the cellular level to identify the changes of spleen tissue description. The histopathological preparation process was done by experts [2], [3].

(3) Examination of CD4 and CD4CD25 Count. The blood collected in vacutainer tubes containing anticoagulant, was ready to be examined by using flowcytometer.

(4) Data Analysis. The statistical analysis on the results of the study used different methods for different data types. Data on the number of peripheral blood cells and th and treg lymphocytes of CD4 and CD4CD25 among the groups and among the periods were analyzed by using ANOVA and Tukey test to analyze the significance differences among the means of the data. The histopathological differences were descriptively analyzed.

## III. Results and Discussion

### A. Results

The DMBA dose of 1 × 15 mg/mouse or 10 × 20 mg/kgbw reduced total leukocytes. The decrease of total erythrocytes and platelet was more visible in repeated dosing. The application of BCSO was able to inhibit the decrease of total leukocytes, erythrocytes and platelets, either in a single dose or repeated administration of DMBA. The results of BSO application and DMBA in percentage of peripheral blood component percentage are shown in Table I.

TABLE I. The Percentage of Peripheral Blood Component SD Rat DMBA-Induced 1 ×15 mg/Mouse (DMBA15) and 10 ×20 mg/kg (DMBA20) with or without Administration of BCSO 0.25 ml/kg

| Groups | N | Leukocytes number | Erythrocytes number | Platelets number | Hemoglobin number | Percentage of neutrophils | Percentage of lymphocytes |
|---|---|---|---|---|---|---|---|
| Normal | 5 | 7.82±3.25 | 7.66±0.37 | 930.00±230.65 | 14.52±0.37 | 16.36±2.03 | 83.64±2.03 |
| DMBA15 | 5 | 4.02±0.79 | 7.18±0.28 | 919.60±237.33 | 14.36±0.22 | 15.48±4.56 | 83.58±3.19 |
| DMBA20 | 5 | 4.62±0.82 | 6.19±0.26 | 530.00±211.31 | 12.70±0.39 | 20.00±3.96 | 79.80±3.66 |
| DMBA15BCSO | 5 | 7.68±1.98 | | 877.40±110.21 | 14.20±0.52 | 15.94±5.60 | 84.06±5.60 |
| DMBA20BCSO | 5 | 7.46±2.52 | 7.66±0.37 | 886.20±177.02 | 12.68±1.40 | 16.64±4.52 | 83.36±4.52 |

According Schalms Veterinary Hematology the normal value of lymphocytes is 81.3 ± 5.1%. Based on the average number of lymphocytes, it was identified that repeated exposure to DMBA more reduced the percentage of lymphocytes in the treatment groups. The normal value of monocytes according to Schalm's Veterinary Hematology is 2.3 ± 1.6%. According to Schalm's veterinary hematology the normal value of neutrophils is 15.4 ± 4.5%. Based on the present study, the number of neutrophils significantly increased from the normal values stated in the literature.

The 1 × 15 mg/mouse or 10 × 20 mg /kgbw DMBA doses provid effects on the reduction of the absolute number of lymphocytes and neutrophils in the peripheral blood of tested animals. The lowest lymphocyte count was indicated in the group receiving repeated doses of DMBA among the groups of tested animals. The application of BCSO was able to inhibit the DMBA exposure-caused decrease of lymphocyte count ($p$<0.05). The condition of spleen of mice is presented in Table II.

TABLE II. THE SPLEEN WEIGHT, PERCENTAGE OF SPLEEN CULTURE CD4 LYMPHOCYTE, SPLEEN PERCENTAGE OF MICE IN ONE GROUP UNDERGOING EXPANSION IN THE WHITE PULP AREA IN DMBA-INDUCED SD MICE

| Groups ($n$=5) | Mean spleen weight (mg) | Σ Spleen | Persentage (%) |
|---|---|---|---|
| Normal | 0.24±0.035 | 4 | 80 |
| DMBA15 | 0.16±0.062 | 1 | 20 |
| DMBA20 | 0.23±0.032 | 1 | 20 |
| DMBA15BCSO | 0.29±0.039 | 3 | 60 |
| DMBA20BCSO | 0.27±0.035 | 4 | 80 |

The spleen of normal condition was most commonly seen in normal control group. This indicated that the groups did not undergo body mechanism to produce antibodies, and one of which was seen from the microscopic description on the spleen characterized by the widespread areas of white pulp because of the absence of lymphocyte cell growth. Spleen undergoing the expansion showed active germinal center, which was characterized by increasing number of lymphocytes [2], [3], [13], [15].

Based on the histopathologic description, in general it was showed that the administration of BCSO affected the immune activity in the germinal center of the white pulp. In the present study, no significant increase in spleen weight was detected. According to the theory, the spleen weight will increase when infection and immune activity occur. In general, the result showed that the BCSO application with the doses of 0.25ml/kgbw/day was the dose showing the greatest expansion of the white pulp characterized by the higher percentages of the spleen undergoing expansion in the area of white pulp [7]-[9].

Total lymphocytes of CD4 and CD4CD25 in the spleen of DMBA-induced SD mice have exemined. The effects of DMBA administration of the higher doses of 1 × 15 mg/mouse and the lower repeated doses of 10x20 mg/kgbw in SD mice on the CD4 percentages are shown in Table III.

TABLE III. CD4TH AND CD4CD25 TREG COUNT IN 5 KIND GROUPS

| Groups ($n$=5) | CD4 account | CD4CD25 account |
|---|---|---|
| Normal | 30.20±5.7 | 2.10±0.50 |
| DMBA15 | 21.37±1.5 | 1.23±0.02 |
| DMBA20 | 23.29±4.2 | 1.24±0.06 |
| DMBA15BCSO | 26.01±3.5 | 6.69±0.30 |
| DMBA20BCSO | 32.92±6.3 | 4.57±1.32 |

The DMBA dose of 1 × 15 mg/ mouse or 10 × 20 mg/kgbw generally suppressed total CD4 of spleen lymphocyte cultures ($P$ > 0.05). The BCSO application was able to suppress the decrease in the CD4 percentage

The effects of DMBA administration at the higher doses of 1 × 15 mg/mouse and the lower repeated low of 10 × 20 mg /kgbw in SD mice on the CD4CD25 percentages were obtained in the study. The DMBA dose of 1 × 15 mg/mouse or 10 × 20 mg/ kgbw in general lowered the percentage of CD4CD25 although it was not statistically significant ($p$ > 0.05). The administration of BCSO is proved to prevent the decrease in the percentage of CD4CD25 lymphocytes.

### *B. Discussion*

The regimen of DMBA diol epoxide has been proved immunosuppressive by suppressing the activity of lymphocyte proliferation and inhibits bone marrow hemopoiesis. The lower percentage of CD4 lymphocyte culture and the increasing of CD4CD25 percentage is consistent with the immunosuppressant activity of the DMBA diol epoxide regimen. Gao et al. [3] further has proved that the active DMBA regimen in addition to suppress lymphocyte proliferation activity also inhibits the differentiation and promotes apoptosis [3], [11], hence, it may explain the phenomenon identified in this research [3]. DMBA diol epoxide active regimen can suppress bone marrow hemopoiesis process resulting in inhibited formation of blood cells [1]-[3], [10], [11]. This is consistent with the results of studies in which the group with single or repeated exposures to DMBA show a lower total leukocytes and lymphocytes compared to the normal one. The repeated exposure to DMBA produces more severe effect on the process of bone marrow hemopoiesis than the single exposure [5], [10], [16].

Black cumin oil contains aromatic oils, trace elements, enzymes, fatty acids, vitamins and minerals, including omega 3 and omega 6. Nigellone is thymoquinone form of a polymer that has the ability to inhibit the enzyme cyclooxygenase and arachidonic lipooxygenase metabolism. Lipooxygenase catalyzes the formation of leukotriene from arachidonic acid, which serves as an inflammatory and allergic mediator. Thymoquinone has also antioxidant, anti-infective and antihistamine effects. In addition, the active ingredient of black cumin also stimulates the immune system [6]-[8].

T helper cell (CD4Th) serves to recognize the presence of antigens and regulate immune responses. The CD4Th0 lymphocytes activated by the presence of the antigen will differentiate into T lymphocytes CD4Th1. Cytokines produced by CD4Th1lymphocytes can increase the effectiveness of effectors, TCD8 lymphocytes, NK cells and macrophages. In contrast to T lymphocytes CD4Th1/th2 serving as a regulator of specific cellular immune response, T cells of CD4CD25 are known as Tregulator cell (Treg) to activate the auto tolerance to self and hence self-destruction is prevented. In addition, the increasing percentage of CD4CD25 may prevent autoimmune reaction [4], [17].

## IV. Conclusion

The result of this research indicates that exposure to DMBA has been proved to reduce the number of leukocytes, particularly lymphocytes, in which the DMBA administration of repeated doses provides a greater immunosuppressive effect compared to the single dose administration. The administration of 0.25 ml/kgbw/day BCSO may decrease the effects of immunosuppressive and hematotoxic of DMBA.

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