

HASIL CEK_Ethanollic Extract of Black Cumin Seed Reduced Radical Reactive from Dimethylbenzanthracene Compounds

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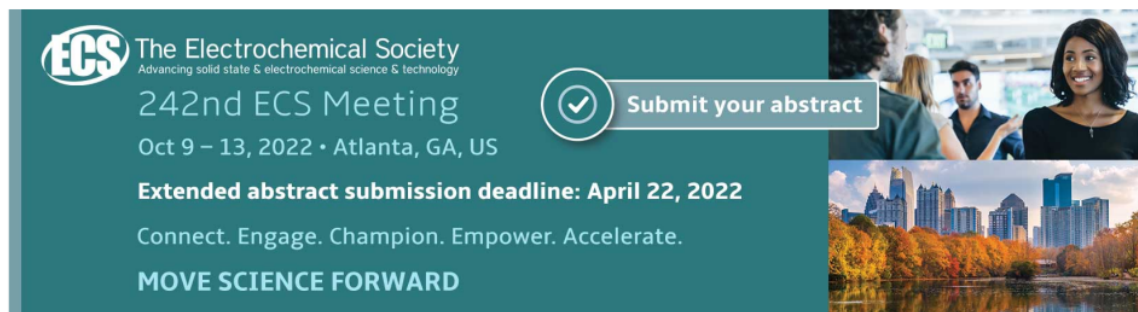
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Ethanollic Extract of Black Cumin Seed Reduced Radical Reactive from Dimethylbenzanthracene Compounds

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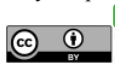
Abstract. Dimethylbenzanthracene is a compound that is radical reactive, genotoxic, and immunotoxic. Black cumin seeds have antioxidative properties. The study aimed to determine the effect of the black cumin seed ethanollic extract as an antioxidant by observing the secretion activity of ROI and NO peritoneal macrophages in Sprague Dawley (SD) rats induced by DMBA. This controlled experiment used 30 male SD rats. The test animals were randomly divided into six groups. Group I was given standard food and drink. Group II was given DMBA. Groups III, IV, and V were given EEBCS doses of 5, 25, and 125 mg orally for seven weeks, respectively. Group VI was given Imboost. From week 3 to week seven, all test animals were induced 10x20 DMBA, 2x per week. At week 16, all test animals were sacrificed and isolated and cultured peritoneal macrophage cells. The activity of ROI secretion and NO macrophage secretion was tested. Data on ROI secretion and NO levels were tested for ANOVA, followed by LSD test with a confidence level of 95%. The results showed that DMBA induction increased ROI secretion by macrophages and decreased NO. EEBCS secretion decreased ROI secretion and grew NO. The DMBA group had the lowest NO levels. This study concluded that EEBCS at doses 5 and 125 reduced the flow of ROI peritoneal macrophages in SD rats induced by DMBA but reduced NO levels.

Keyword: Ethanollic Extracts of Black Seeds, macrophage, ROI, DMBA, NO

1. Introduction

Oxidative stress has had a broad impact on public health status. Dimethylbenzanthracene (DMBA) is a polycyclic aromatic hydrocarbon, carcinogenic, genotoxic, and immunotoxic [1][2]. DMBA is found in vehicle smoke, kitchen smoke, and cigarette smoke. Liver enzymes will metabolize DMBA inhaled while breathing in the body into reactive radicals [3]. DMBA induction has been shown to trigger the creation of DNA adducts, suppressing lymphocyte proliferation activity, and macrophage phagocytosis [4]. Macrophages as effector cells of the non-specific immune system. The macrophage role is in the cellular immune response. Macrophage plays a role in inflammatory processes, tissue damage, tissue reorganization, killing tumor cells, and bacteria. The mechanism of killing tumor cells by macrophages is thought to be the same mechanism as the pathogenic microbial killing mechanism, namely releasing Reactive Oxygen Intermediates (ROI) and nitric oxide (NO). ROI are very toxic to bacteria [5]

Black cumin seeds contain evaporated oil, non-volatile oils, unsaturated, and saponins. The bioactive content of BCS has antioxidant, immunomodulatory, and chemopreventive activities [6]. Thymoquinone reduces the inflammatory reaction in asthma and exposure to toxic substances [7] by



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decreasing the secretion of histamine by mast cells and by increasing the in vivo phagocytosis function of macrophages[8]. At doses of 5 mg/kg BW, 25 mg/kg BW, and 125 mg/kg BW, ethanol extract of black cumin seed has been shown to inhibit and decrease the formation of ca mammae in female SD rats induced by intragastric DMBA 10x20mg/kg BW [3]. The research aim is to determine phagocytosis, ROI secretion, and NO secretion by the macrophage.

2. Research methods

2.1. Materials and tools

Materials for testing phagocytic activity are black cumin seeds; 96% ethanol; IMBOOST (Soho); physiological NaCl; absolute methanol, aqua, aqua bidest, RPMI 1640 (Sigma), FCS 10%, Phosphate Buffernet Saline (PBS), Nitro Blue Tetrazolium (NBT), corn oil (Tropicana), NaOH 1 N, and Phorbol 12-Myristate 13 Acetate (PMA). DMBA (7,12-dimethylbenz (α) anthracene) (Sigma Chem) for making cancer models.

2.2. Research procedure

a. Preparation of ethanolic extract of black cumin seed (EEBCS)

The ethanol extract of black cumin seeds was carried out in the Phytochemical Pharmacognosy Laboratory of the Faculty of Pharmacy, Ahmad Dahlan University.

b. Treatment and administration of DMBA induction

In a well-ventilated room, the rats were housed, given food in pellets and enough aqua drinks. Until surgery, animals were used to cages for one week. Three levels, namely 5, 25, and 125 mg/kg BW, were used in the ethanol extract dosage of the black cumin seeds used in this analysis. The dosage of Imboost used was one teaspoon (5 ml) with a human to mouse conversion ratio of 0.018 / 200gBW. DMBA is dissolved in corn oil at a dose of 20 mg / Kg BW and is always made fresh before being given to the test animals. Dissolution of Vortex-assisted. The 30 single-month-old female rats were randomly divided into six groups. The group consisted of 5 rats each. The solvent control group provided corn oil was Group one. At a dose of 0.0014 ml/kg BW rats, Group VI was a positive control group using Imboost®. Group II was the group in which DMBA in corn oil was induced at a dosage of 20 mg/kg BW 10 times orally, i.e. twice a week for eight weeks at the age of 1.5 months. 14 days earlier, only control feed was given to the rats. Ethanol extract from black cumin seeds was administered to Group III-V at doses of 5, 25 and 125 mg/kg BW/day for 14 days prior to initiation (DMBA administration) and during initiation. Treatment was carried out orally for each party..

c. Culture, and phagocytic activity test of macrophages

At the 16th week of treatment (2 weeks of production, five weeks of DMBA induction, and eight weeks of post-induction EEBCS), an autopsy was performed. By pushing on the inner hole with two fingers, peritoneal fluid is separated from the peritoneal cavity. The aspirate was centrifuged for 10 minutes at 1,200 RPM, 4°C. The supernatant was discarded, with RPMI containing 10 percent FCS resuspended from the pellets. A hemocytometer counted the number of cells, then applied a complete medium to achieve a cell suspension with a density of 1×10^6 / mL. In a microculture of 24 wells that had been handled with round coverslips, the cell suspension was cultivated. 200 microliters (2×10^4 cells) filled each well. Cells were incubated for 30 minutes in a 5 percent, 37°C CO₂ incubator, then 1 ml of complete medium per well was added and set for 2 hours. With 1 mL RPMI per well the cells were washed 2 times and incubated in full medium for up to 24 hours. [3].

The latex particles were resuspended in PBS to obtain a concentration of 2.5×10^7 / ml. Macrophages that have been cultured the day before were washed twice with RPMI medium, then added latex suspension 200 μ L/ well incubated in a 5%, 37°C CO₂ incubator for 60 minutes. Then the cells were washed with 3x PBS, dried at room temperature and fixed for 30 seconds with absolute ethanol. It is covered with 20 percent Giemsa after drying. The percentage of macrophage activity and the number of phagocytised latex particles were counted from 100 cells examined by a light microscope with 400x magnification. [9][10].

d. ROI secretion and NO secretion test

The capacity of peritoneal macrophages to secrete ROI was calculated by the NBT reduction assay. NBT can be oxidized in the presence of O₂ to form undissolved precipitates of formazan. The macrophage cell culture was stimulated to induce superoxide anion secretion with Phorbol 12 Myristate 13 Acetate (PMA) with a final concentration of 125 ng/ml. Macrophages cultivated for 24 hours were washed twice with a medium RPMI, then added 500 µl of NBT solution 1 mg/ml of PBS containing 125 ng/ml of PMA, and incubated for 60 minutes in a 5% CO₂ incubator, 37°C. Then the cells were washed with 3x PBS, dried at room temperature and fixed for 30 seconds with absolute methanol. It is dissolved with a 2 percent neutral red solution after drying. The percentage of macrophage cells exhibiting reduced NBT was measured under a 600x magnification light microscope from approximately 100 cells examined. Calculation of the macrophage cell rate, which showed a 3x reduction in replication of NBT [3].

e. Test the NO levels in the macrophage supernatant using griess' solution

The solution Griess A consists of 0.1 gram of ethylenediamine hydrochloride (Sigma N 5889) N-(1-naphthyl) in 100 mL aqua dest. The solution of Griess B consists of 1 gram of sulfanilamide (Sigma S9251) in 100 mL of 5 percent orthophosphoric acid (v / v). Store, shielded from light, at 0-4 ° C. In brief, the method for measuring NO levels is 10 mM nitrite standard: 69.0 mg sodium nitrate is dissolved in 100 mL aqua dest. Store at 0-4 ° C, shielded from light, make regular solutions between 0-50 uM nitrite from this inventory. In a 24-well microtiter plate, a total of 100 µL of standardized macrophage and nitrite culture supernatant specimens were placed. When less than 100 µL of the example was used the amount of water or medium was increased to 100 µL. 100 µL of Griess solution was added and incubated for 15 minutes at room temperature before it changed color. Measure the absorbance using an ELISA reader after all samples and standards have been placed into the microtiter plate. For example, the absorbance size and norm is 540 nm [3][5].

2.3. Data analysis

Annova carried out various tests between groups with a confidence level of 95 percent for mean phagocytosis activity, percentage of ROI and NO secretion.

3. Results and discussion

3.1. Effects of the EEBCS on macrophage phagocytosis and ROI secretion

Black cumin seeds (BCS) are obtained from traditional medicine shops in Semarang, Central Java. BCS identification was carried out at the Biology Laboratory of Gadjah Mada University, Yogyakarta. The authenticity of the test material in this study. It is proven by the issuance of a certificate of authenticity of the material. Extraction by the maceration method yielded a 25mg yield. The macroscopic picture of the ethanol extract of BCS obtained from the extraction process is brown, oily, and thick with a distinctive smell of essential oils. Macrophage phagocytic activity is measured by the ability of macrophages to consume latex particles. Figure 1 shows the definition of macrophages that eat latex and that do not eat latex.

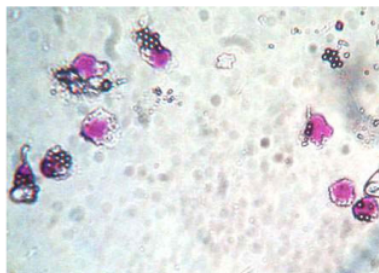


Figure 1. The latex-eating macrophages in the Imboost group are shown with arrows. Giemsa stain and 400x magnification (indicated by arrows).

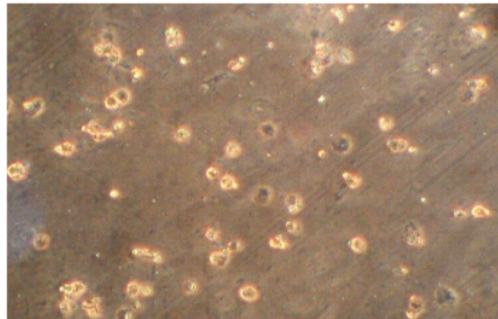


Figure 2. Macrophages secreted ROI in the treatment of 125 mg of black cumin seed ethanol extract with a magnification of 600 x (shown with arrows).

Based on Table 1, it is known that the highest number of latex ingested macrophages was in group VI (Imboost) of 92.00%. Macrophage phagocytosis activity in The DMBA (a negative control group) compared to the EEBCS at a dose of 5, 25, and 125 mg / Kg BW showed a significant difference ($p < 0.05$). The group with an enormous amount of latex ingested by macrophages was group VI (Imboost) (641.00). The lowest was the solvent control group (427.33). DMBA induction increases macrophage phagocytosis activity as well as EEBCS and Imboost administration. Group II phagocytosis activity (DMBA) was higher than the solvent control group.

Table 1. Number of latex, percent of macrophage phagocytosis activity, and index of SD mice macrophages induced by DMBA and giving EEBCS

Group (n)	The amount of latex that is phagocytosed (Mean \pm SD)	Macrophage phagocytosis activity (mean)	Phagocytosis index
Corn oil group (6)	427,33 \pm 31,37	77,67 \pm 2,52	5
DMBA group (6)	446,33 \pm 45,76 ^b	75,67 \pm 2,52 ^b	6
EEBCS 5 mg/kgBW group (6)	515,67 \pm 34,93 ^a	80,33 \pm 0,58 ^a	6
EEBCS 25 mg/kgBW group (6)	580,67 \pm 15,18 ^a	85,67 \pm 2,31 ^a	7
EEBCS 125 mg/kgBW group (6)	586,00 \pm 21,07 ^a	87,00 \pm 1,00 ^a	7
Imboost group (6)	641,00 \pm 8,72 ^a	92,00 \pm 1,00 ^a	7

Note : b = Not significance compared to corn oil group

a = Significance : Significant difference (significantly different) compare to DMBA group

EEBCS : Ethanol Extract of Black Cumin Seed

The phagocytosis activity of the treatment group (III, IV, and V) was higher than the DMBA group, and the highest was the positive control group. Phagocytosis index is used to measure the level of phagocytic activity of macrophages. The greater the phagocytosis index is, the higher the phagocytosis activity of the macrophages. From the calculation of the phagocytosis index, corn Oil groups had a phagocytosis index of 5. Imboost group had a phagocytosis index of 7; negative control (DMBA) had a phagocyte index of 6. In the EEBCS group, the dose of 5; 25 and 125mg / kg BW, respectively, having a phagocyte index of 6; 6 and 7.

3.2. Effects of the EEBCS on macrophage ROI and NO secretion

Figure 1 shows macrophages with formazan salt deposits in them (macrophages that secrete ROI). Table 2 indicates that the peritoneal macrophages of mice were able to emit ROI from all treatment classes. The solvent control group had the lowest ROI secretion percentage (29.67 ± 2.89). DMBA induction improved macrophage ROI secretion; the DMBA group's ROI secretion was shown to be higher than the solvent control group (43.00 ± 2.65 v.s. 29.67 ± 2.89) ($p < 0.05$). The highest percentage of ROI secretion was found in the Imboost category (66.67 ± 5.69 percent). EEBCS administration reduced ROI secretion by SD mouse macrophages induced by DMBA. The ROI secretion activity of the EEBCS group at 1 mg/kg BW, 5 mg/kg BW and 125 mg/kg BW doses was lower than that of the DMBA group and was statistically important. ($p < 0.05$).

Table 2. The mean percentage of macrophages secreting ROI and NO test of SD rats with the administration of ethanol extract of black cumin seeds

Group	ROI (Mean \pm SD (%))	NO (Mean \pm SD (%)) (μ M)
corn oil group (6)	$29,67 \pm 2,89$	$0,266 \pm 0,039$
DMBA group (6)	$43,00 \pm 2,65c$	$0,094 \pm 0,038c$
EEBCS 5 mg/KgBW group (6)	$35,33 \pm 3,79a$	$0,126 \pm 0,032a$
EEBCS 25 mg/KgBW group (6)	$34,00 \pm 4,00a$	$0,217 \pm 0,113a$
EEBCS 125 mg/KgBW group (6)	$33,67 \pm 2,52a$	$0,119 \pm 0,041a$
Imboost group (6)	$66,67 \pm 5,69a$	$0,332 \pm 0,222a$

Note : EEBCS = Ethanol Extract of Black Cumin Seed

a = Significance compare to DMBA group; c = Significance compare to corn oil group

The absorbance results and NO concentration data by macrophages in the peritoneum of female Sprague Dawley rats from each treatment group due to the ethanol extract of black cumin seeds, Imboost®, DMBA, and corn oil can be seen in Table 2. Peritoneal macrophages of mice from all groups were able to secrete nitric oxide (NO), where the positive control group was able to secrete the highest NO compared to the other treatment groups, namely $0.332 \pm 0.222 \mu$ M. The lowest in secreting NO was the negative control group given Imboost®, which was $0.094 \pm 0.038 \mu$ M. In the test group, the order from the highest to the lowest was the dose of 25 mg/kg with NO levels of $0.217 \pm 0.113 \mu$ M, the dose 5 mg/kg BW with levels $0.126 \pm 0.032 \mu$ M, a dose of 125 mg/kg BW and levels $0.119 \pm 0.041 \mu$ M.

The results of several studies that have been conducted have shown that black cumin seeds have an antioxidant effect. The black cumin seed oil emulsion at a dose of 400 mg/100gBW was able to inhibit DMBA-induced NO (Nitric Oxide) production in mice[11]. NO is used as a marker compound (marker) for oxidative stress, a condition where free radicals accumulate and the inability of antioxidants to eliminate the accumulated free radicals causing an imbalance in the production of reactive oxygen species [12]. Thymoquinone reduces tumor incidence and fibrosarcoma weight. The possible mechanism of action of thymoquinone is an antioxidant activity [13]. Thymoquinone acts more as a superoxide anion scavenger than hydroquinone. Black cumin extract can affect the levels of GSH in the lungs and liver of Wistar rats, namely the higher the dose of black cumin (600mg / KgBW, 1200mg / KgBW, 2400mg / KgBW), the higher the GSH (glutathione) level compared to the group that only got cigarette smoke. GSH is a tripeptide compound that plays a vital role in the body's defense, which functions as an antioxidant[13].

4. Conclusion

The ethanol extract of black cumin seeds increased the activity of phagocytosis and NO secretion, and decreased the activity of ROI secretion caused by DMBA in mice. The author would like to thank the managers of LPPT UGM and UPHP who have permitted to carry out research and use of existing laboratory facilities. The author also thanks to the UGM Pharmaceutical Biology Department for identifying black cumin seeds.

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