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The Effects of SNEDDS (*Self-Nano Emulsifying Drug Delivery System*) Black Cumin Oil Seeds (*Nigella sativa L*) to the Histopatology of Lung Organs of The Sprague Dawley Rats Induced by DMBA

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***Abstract*—Lung cancer is the cancer that caused death, especially for men in Indonesia. One of the compounds that causes cancer is 712-Dimethylben(a)anthracene (DMBA). Black cumin seed oils (BCSO) is able to reduce the damage of lung cells to the DMBA-induced rat. To improve total medicine that could arrive and react at the cancer cell, it's necessary to make self-nanoemulsifying drug delivery system (SNEDDS). The aim of this research is to know the effect of SNEDDS BCSO into of histopathological lung organ of the Sprague Dawley (SD) rat that's already DMBA-induced. The making of SNEDDS is by using BCSO, tween 80 and PEG 400. Treatment was done in vivo towards 25 SD male rats, which are divided to 4 groups. The first group act as the baseline, the second one are given with the DMBA, the third group was given BCSO as equivalent as thymoquinone 6.8 mg/KgBW, and group 4 was given BCSO SNEDDS as equivalent as 6.8 mg/KgBW of thymoquinone. Next, the rat discharged and lung organs are taken then being H & E painted. Based on results of histopathology lung in a descriptive manner, there's no specific differences of histopathology between the group treatment. Next, scoring is done based on alveolar category damage. After that, was done the Kruskal Wallis test, which obtained P value = 0.801. Because of the p value was bigger than 0.05, then this statistical test didn't show any significant difference at each of the group. This research concludes that there is no influence from BCSO SNEDDS to histopathology of DMBA-induced lung compared with BCSO administration only.**

Keywords— SNEDDS, nigella sativa, DMBA, histopathology, lung

I. INTRODUCTION

Lung cancer is one type of cancer that has a high level of incidence in the world. In Indonesia, lung cancer is the main issue of dying men and the reason behind that is smoking [1]. Cause of the occurrence of cancer are various, including environment factor and genetics. Cancer can also happen with existence of carcinogen such as 7,12 – dimethylbenz (a) anthracene (DMBA) which causes cancer in the breast, lungs, lymphoid, stomach and skin [2].

Research shows that N. sativa is able to reduce damage to the lung cells of the DMBA-induced rat. Inflammation that occurs is periartritis, which had the possibility as an early formation of lung cancer in mice consequence of DMBA induction . Inflammation that occurs in lung cells in the rat is able to get blocked by existence extract chloroform seed N. sativa. This is seen in it's histopathology picture. Lung cells in the group DMBA controls experience periartritis while in the extract group, chloroform cells did not experience periartritis [3].

The biggest content of essential oil is thymoquinone (27-54%). Thymoquinone is one compound from the benzoquinone group, a compound group which is generally have biological activity as anti-inflammatory [4]. Thymoquinone is considered effective as an antioxidant, anticarcinogenic and antimutagenic agent [5]. Thymoquinone is tend to be hydrophobic that it's has a poor solubility in the water [6].

On the other side, the cancer cell have ability to efflux foreigner compounds which will enter the cancer cell, the consequences therapy cancer is not in maximum capability. Thus, it's needed to do some effort to increase total medicine that can arrive at the cancer cell, one of the method is with the SNEDDS. SNEDDS is oil mixture, surfactant, co-surfactant, and medicine in a smooth form that will form nanoemulsion of oil in the water if it got a contact with water phase [7], because of that, large interface oil/water increases, followed by solubility enhancement, then it will improve bioavailability. Some SNEDDS components, in particular, surfactant such as tween 80, cremophore, labrasol potentially inhibit efflux [8].

Wahyuningih and Widyasari [9] have been formulating SNEDDS (BCSO) using tween 80 as surfactant and PEG 400

as co-surfactants. However, it is not yet known the effect to picture organ histopathology of the lung DMBA-induced animals. The primary aim of this study was to know the effect of BCSO SNEDDS on picture pulmonary organ histopathology SD mice induced by DMBA.

II. METHOD

A. Materials

Material used for the making of SNEDDS is cumin black oil, tween 80 as surfactant and PEG 400 as cosurfactant. Activity test in in vivo way needed 7,12 dimethylbenz (a)anthracene (DMBA) (Sigma Aldrich) with the dose of 20 mg/KgBW dissolved in corn oil, distilled water, 10% formalin, and physiological NaCl 0.9%. Hematoxylin and eosin as histopathology painting.

B. Preparation of DMBA solution

According to [10], DMBA carcinogens solution can be made with corn oil as solvents. DMBA solution is then administered orally to the test animals at a dose of 20 mg/KgBW for 5 weeks with twice-weekly administration. DMBA solution is made by dissolving a number of DMBA. According to the dosage, with corn oil until the solution is obtained with a concentration which if given to the test animal, it will get a volume of administration between 0.5-1.5 mL. DMBA solutions are always made new, before being given to test animals [11], [12].

C. Preparation of BCSO SNEDDS

Surfactant (tween 80) and co-surfactant (PEG 400) mixed using a vortex (Thermolyne Type 16700 Mixer) with maximum speed for 1 minute. BCSO is added little by little while continue to be mixed for 2.5 minutes. The BCSO, tween 80 and PEG 400 mixed by using ultrasonic (Elmasonic S 30 H) for 1 hour. The composition of the formula is BCSO 18.75%, tween 80 72.15% and PEG 400 9.1% [9].

D. Determination of the Transmitan of the SNEDDS

SNEDDS formula which was already sonicated then will be taken 100 µL then mixed with distilled water up to 5 mL, then vortex for 1 minute. The percentage of the transmission read in the wave length of 650 nm [13] by using spectrophotometer (Shimadzu UV-1800), replication 3 times.

E. Determination of Emulsification Time of the SNEDDS

Emulsification time is done with using disolution tester apparatus II (Erweka) with medium distilled water. A total of 500 mL distilled water conditioned on the dissolution tester at 37°C. After temperature of the distilled water reaches 37°C, SNEDDS MBJH were dropped as much as 1 mL of the same with the turn around paddle at a speed of 100 rpm. Noted the time required to SNEDDS MBJH truly dissolved in distilled water medium, replication 3 times [7].

F. In vivo Study Protocol

The study protocol was reviewed and approved by the Ethics Committee (reference number 011610144), Universitas Ahmad Dahlan. All SD rats experience adaptation in cage trial for two weeks to homogenize their way of life, eating habits, and cage trial conditions. All of the rats was given commercial food and water by ad libitum (free). Mice bedding (pedestal) is chaff done replacement twice a week. Healthy male rats were 25 and divided in a manner random in 4 cage. Every cage contains 5 rats. Group treatments are:

Group I (normal). Normal group is a group mice only fed with standard and water quantity during the research period.

Group II. This group were given DMBA treatment with dose of 20 mg/KgBW. DMBA is given and done for 5 weeks and as much twice in a week.

Group III. This group of mice were given the BCSO treatment. The provision of BCSO is carried out once in a day for 2 weeks. Dose of BCSO given is equivalent thymoquinone 6.8 mg/KgBW. After 2 weeks, the rat was DMBA induced for 5 weeks with DMBA administration twice a week.

Group IV. This group of mice were given the treatment of BCSO SNEDDS. The giving frequency of the BCSO SNEDDS is done once in a day for 2 weeks. BCSO SNEDDS dose given is equivalent thymoquinone 6.8 mg/K gBW. After 2 weeks, the rats were DMBA induced for 5 weeks with DMBA administration twice a week.

G. Histopathology protocol with Hematoxyline and Eosine Method Painting

Histopathology analysis done with observation circumstances pulmonary organ cytology from results H & E staining and tumor or cancer severity that occur. Microscopic analysis was carried out by observing the cellular carcinogenicity of the tissue of the examined, then the scoring was based on the level pulmonary alveolar damage. Assessment done with using ordinal scaled categorization in 5 fields look at with score 0-3 for look level pulmonary alveolar damage [14]:

Score 0 =Nothing happened in the histological structure (normal)

Score 1 =pulmonary alveolar damage 0-30% (damage light)

Score 2 =pulmonary alveolar damage 31-60% (damage medium)

Score 3= pulmonary alveolar damage >60% (damage heavy).

H. Analysis and Evaluation Results

Data results are analyzed using a statistics data processing program. Because of research's a comparative categorical more from two group not in pairs then used the Kruskal Wallis test [15].

III. RESULTS AND DISCUSSION

A. Characteristics of BCSO SNEDDS

Reading results of the transmit an obtained, an average of 96.42%. Percent transmittance of SNEDDS BCSO made by [9] amounted to 95.49%. Percent the transmit formula that made have value slightly more big from the reference formula,

this shows estimate droplet size has been reached nanometer size because has been close to 100%.

Emulsification time obtained an average of 44.74 seconds. Compared with emulsification time from [9] which is 19.94 seconds, the results obtained has the time emulsification longer but less from 1 minute, so that said SNEDDS that was made were able to form emulsion after contact with fluid gastric.

B. Histopathology of Pulmonary Organ Rat

Histopathology analysis done for comparing preparations histopathology deliver group, i.e. group control and group treatment. All group experienced cell change that can be seen in Table I. Identify applicable funding agency here. If none, delete this text box.

Inspection results of histopathology of the lung seen existence inflammation in the area interstitial lung and BALT activated that occur in all group including normal group. In the case of the normal group bronchopneumonia suppurative in 1 test animal. Lung Cell Normal (LCN) occurred in the BCSO group of 1 test animal.

TABLE I. HISTOPATHOLOGY OF RAT LUNGS SD IN VARIATION TREATMENT

Rat Number	Normal group	DMBA Control	BCSO	BCSO SNEDDS
1	PI (++), BALT is active	PI (+), BALT is active	-	PI (++), BALT is active
2	BPS	PI (++)	PI (+), BALT is active	-
3	PI (++), BALT is active	PI (+)	TAP	PI (+)
4	PI (++), BALT is active	PI (++)	PI (++)	PI (++)
5	PI (++)	PI (++), BALT is active	PI (++), BALT is active	PI (++), BALT is active

[a.] Information:
BALT: Bronchus Associated Lymphoid Tissue,
BPS: Bronchopneumonia Suppurative,
PI: Interstitial Pneumonia,
TAP: No change.

Normal lung has characteristic features bronkhiolus have epithelium shaped cuboid or columnist with trophy-shaped cell, no necrosis, erosion, and proliferation, the lumens looked blank and does not contain blockage. Normal alveoli, the epithelium were in the form of squamosa and does not experienced thickening or inflammation and alveolar lumen was not experience any narrowing and blockage. Interalveolar septa didn't looked like thickening [16]. This is not corresponding with the theory where the histopathology normal groups experienced inflammation in interstitial area and experienced suppurative bronchopneumonia. Microscopic normal lung can see in Figure 1.

All parts of the respiratory system can experience inflammation and infection. Changes in the lungs can occur in the alveoli, interstitial tissue, bronchi and bronchioles. Inflammatory tissue response that is protective against injury or tissue damage and reduced function destroys both the agent that causes injury or tissue injury [17]. Interstitial inflammation occur in interalveolar septa, causing thickening of the alveolar septa and narrowing walls. Some other alveoli will be widened in order to fulfill the supply shortage of oxygen in the alveoli due to narrowing of the right because inflammation [18].

Interstitial pneumonia is an inflammation that occurs in parts interstitial alveoli that covers network interstitial and alveolar parenchyma. Interstitial lungs thickening happens because proliferation cell pneumocytes type II, macrophages , and infiltration cell lymphocytes [19]. Infiltration cell inflammation lymphocytes in interstitial lungs caused by several factor including chronic infection by infectious agent, neoplasia, and stress conditions [20]. Inflammation that is happen could cause by factors environment a water sanitation, influence of a cage that doesn't clean, bacteria and viruses. The virus can also be cause inflammatory pulmonary [21]. The virus that causes pneumonia in mice including coronavirus and paramyxoviridae family, including parainfluenza I [22]. Virus transmission through aerosols and contacts exposure to the channel breathing. Intranasal infection results in mild rhinitis and interstial pneumonia [23]. Microscopic interstitial pneumonia in the lungs could see in Figure 2.

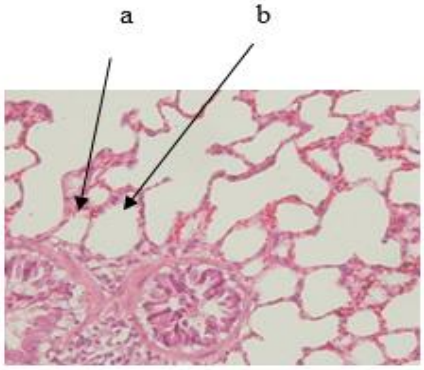

Fig. 1. Microscopic normal lung occurs in the BCSO group with H & E coloring, 200x magnification, a. interalviolar septa, b. alveoli.

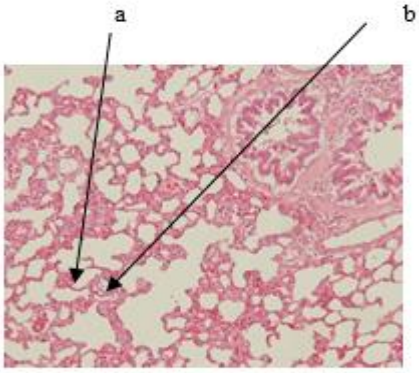

Fig. 2. Microsopic interstitial pneumonia in the lungs group BCSO with H & E coloring, 100x magnification, a. narrowing of the alveoli, b . thickening of the interalviolar septa.

Bronchopneumonia suppurative could be marked by infiltration of neutrophils and macrophages in the lumen, mucosa and tissues in around bronchus. Inflammation occurs in the parenchyma of the lungs, bronchioles or bronchi then called bronchopneumonia. Formation suppurative inflammation is caused by extracellular bacteria with stimulate reaction inflammation that causes destruction in infection place. Extracellular bacteria could live and develop multiply on outside of cell as in circulation, lumen breath channel, connective tissue [24]. Microscopic suppurative bronchopneumonia in the lung could see in Figure 3.

Bronchus Associated Lymphoid Tissue (BALT) is one of the lymphoid system defense Local respiratory organs contained in the bronchi. According to [25], incoming antigens to in lungs will get contact with cell lymphoid which will induces and activates BALT. The BALT is active is caused by a possibility, Stimulation could be caused by antigens that cause inflammation chronic. BALT consists on cells lymphoid which is responsible answer to response cell-mediated immune [26]. If there's going to happen a stimulation that is chronic, then total cell BALT lymphoid will increase. Increasing density cell lymphoid in BALT will also improve system defense the body. Microscopic results active BALT in the lungs could see in Figure 4.

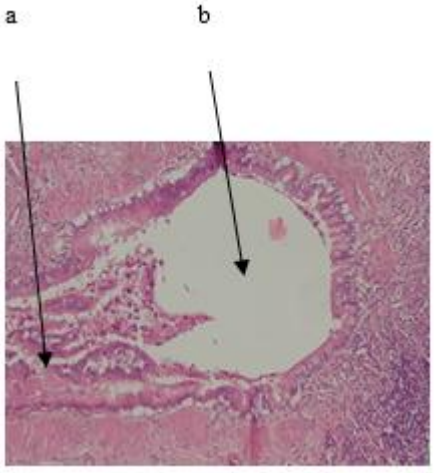

Fig. 3. Microscopic bronchial suppurative pneumonia in the lungs normal group with H & E coloring , 200x magnification , a . pus , b . bronchus.

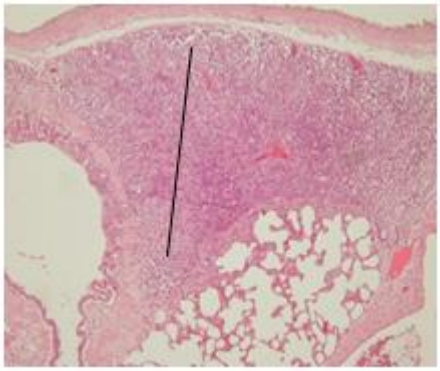

Fig. 4. Microscopic BALT is active in the lungs MBJH group is marked with black line (——) with H & E coloring, 100x magnification.

Giving DMBA dose of 20 mg/KgBW 2 times a week for 5 weeks could not raises cancer lung possibility caused because for the occurrence of cancer lung needs more specific carcinogens to works on lung organs. Initiator usually in the form of the active metabolite or electrophilic. Could react with DNA irreversible, the example is DMBA. Changes that occur in DNA are not always developing to be cancer, because need a promoter. The promoter is substances that must be there is for start occurrence growth cancer. Promoters usually work on specific organs. Next followed by the breeding progression cell in a manner fast and excessive so that formed cancer [27]. Not existence incidence cancer can also influenced by less long time observation remembering cancer is a diseases that are linearly proportional to time and dosage gift compound carcinogens that haven't could raises cancer. Carcinogenesis needs accumulated cellular change which needs the right time to get it. The longer the time observation, probability incidence cancer more and more big [28]. In the research of [29], cancer lung happen with DMBA dose single 4.2 mg for 30 days and research [30] proves that DMBA can trigger occurrence cell cancer lung with dose single 4.2 mg/day for 30 days.

Description of histopathology observed is damage alveolar and divided in 4 categories. Fourth category is normal, light damage, medium damage, and the heavy one [17]. Based on 4 categories then, next was done scoring to results picture histopathology each group are presented in Table II.

TABLE II. SCORING OF HISTOPATHOLOGY LUNG OF THE MALE SD RAT AT VARIATIONS TREATMENT

Group	Total Animal Test and Scoring Damage				
	Normal (0)	Light (1)	Medium (2)	Severe (3)	Average
1 (Normal)	0	1	4	0	1.8
2 (DMBA)	0	2	3	0	2
3 (BCSO)	1	1	2	0	1.25
4 (BCSO SNEDDS)	0	1	3	0	1.75

Data obtained is homogeneous one. However not normally distributed. From calculation statistics using the Kruskal Wallis test obtained p value = 0.801 or could concluded there is no any significant difference deliver group treatment. There is no difference histopathology caused variation effect from each test animals, though given with the same treatment.

IV. CONCLUSION

In research this could concluded not there is influence the provision of BCSO SNEDDS to histopathology induced lung DMBA compared with BCSO giving only.

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