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Research Article

Anticancer Activity of *Centella asiatica* Leaves Extract in Benzo(a)pyrene-Induced Mice

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ABSTRACT

Utilization of *Centella asiatica* leaves extract became an alternative to determine its activities. *Centella asiatica* contained of *asiatic acid, madecassic acid, glicosides, asiaticoside, madecassoside*, and some other compound. *Centella asiatica* has the potential as anti-cancer medicine. This research aims to understand the effects of *Centella asiatica* leaves extract on decreasing the number of benzo(a)pyrene induced lung tumor nodules. Then, determine the lung histopathological features of induced mice. This research used 30 of one day old baby mice which divided into six groups. Positive control (group I) was induced with benzo(a)pyrene of 0.2 µmol on day-1; 0.4 µmol on day-7 and 0.8 µmol on day-15 without any extracts. Group II was treated with Tamoxifen and induced with benzo(a)pirene. Negative control (group III) received only DMSO solvent and groups III, IV, V received 250, 500 and 750 mg/kg bw of *Centella asiatica* leaves extract on day-25. The significant difference ($p<0.05$) of *Centella asiatica* leaves extract on the number of benzo(a)pyrene which induced by lung tumor nodules. The treatment with 250, 500 and 750 mg/kg bb doses of extract resulted a non significant different ($p<0.05$) means number of lung tumor nodules. Microscopic examination of lung histopathological features showed a decrease on tumor foci in bronchus, alveolar septum and also inhibit bronchial epithelial cell hyperplasia. Ethanolic extract of *Centella asiatica* could reduce the number of tumor nodules and inhibit the development of benzo(a)pyrene.

Keywords*:* Lung cancer, benzo(a)pyrene, *Centella asiatica*, tumor nodule, hyperplasia

INTRODUCTION

Some cancer disease management efforts still meets many obstacles that lead to a lack in preventing and treating cancer. Treatments that have been made including surgery, radiotherapy radiation and chemotherapy drug consumption[1]. The Surgery which aimed to remove cancerous tissue did not guarantee a cure and there was a tendency for the occurrence of tissue re-multiplication. The application of radiotherapy clearly raised the risk of damage or metastasis of other tissues which surrounding the cancerous tissues. Anticancer chemotherapy has less selective pharmacological effects, adverse side effects, and was reported to be resistance with some types of cancer.

The discovery of medicinal plants which showed pharmacological effects against cancer, was prompting some researchers to explore the bioactive material from the plant. Pre-screening test was including in vitro and in vivo methods to determine the main effect of plant in inhibiting cancer cell. The study was limited on the method and the observation of cancer cell viability was still simple, but the mechanisms of cancer cell growth process was very complex, which in line with the development of knowledge about molecular biotechnology.

Right now, many people turns to use natural ingredients as for cancer treatment. One of it is pegagan (*Centella asiatica*). The previous research of *C. asiatica* with the active compound *asiatic acid* was recommended the use of this plant in the prevention and treatment of cancer[2].

The Examination by using HPLC showed active compound content in the herb *C. asiatica* is terpenes: *asiatic acid*, *madecassic acid*, *glicosides*, *asiaticoside*, and *madecassoside*. The Other active ingredients are *thankuniside*, *isothankuniside*, *brahmoside*, *brahmic acid*, *brahminoside*, *meso-inositol*, *centelloside*, *carotenoids*, *hydrocotylin*, *vellarine*, *tannins* and mineral salts such as potassium, sodium, magnesium and iron. The suspected triterpenoid is a glycoside called asiaticoside which an extraordinary wound healer anti-leprosy[3]. While the compounds that responsible for anti-cancer properties are Asiatic acid[4].

Based on recent research, the content of Asiatic acid extract from *C. asiatica* leaves can inhibit the effects of pro-angiogenic VEGF and the development of glioma on endothelial cell culture models, namely the Human Brain Microvascular Endothelial Cells (HBMEC)[5].

MATERIALS AND METHODS

Tools and materials

The tools used were: glassware, injection syringe 1 ml 30 G (Terumo®, Japan), injection syringe 1 ml 26 G (Terumo®, Japan), oral syringe, flask, pipette volume, scalpel and bladder, surgical scissors, tweezers, wax

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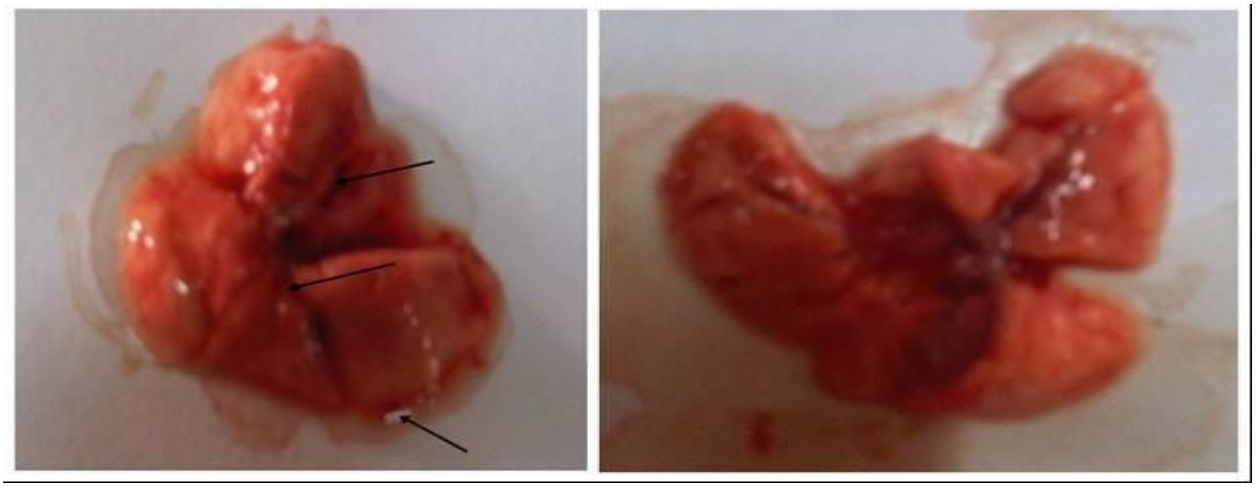

Figure 1: Lung tumor nodules in control treatment group Benzo(a)pyrene (shown in black arrow head)

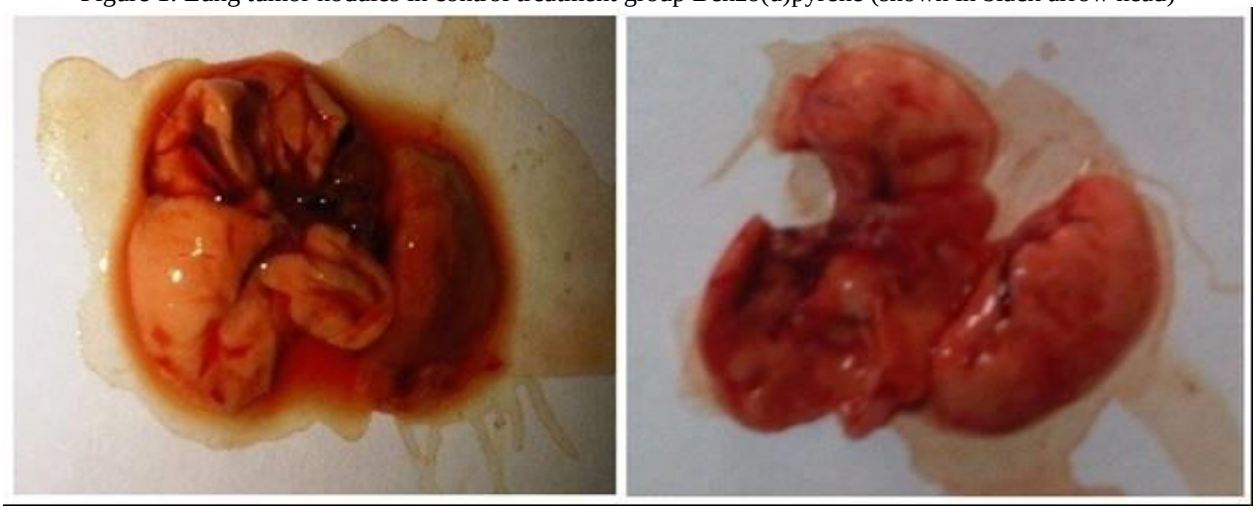

Figure 2: Lung without tumor nodules in negative control group solvent (left) and lung tumor nodules with the addition of *C.asiatica* water extract dose 500mg/kg bw (right).

Table 1: The Average number of mice lung tumor nodules in each treatment group

Treatment group	Number of tumor nodules (Average ± SD)
I. Benzo(a)pirene Control	$5{,}60^{c} \pm 2{,}07$
II. Benzo(a)pyren dan Tamoxifen Control	$0{,}00^{b} \pm 0{,}00$
III. Solvent control DMSO dan CMC Na	$0{,}00^{a} \pm 0{,}00$
IV. Provision of benzo(a) pyrene and water extract *C.asiatica*doseof 250mg/kg bw	$4{,}80^{c} \pm 1{,}64$
V. Provision ofbenzo(a) pyrene and water extract *C.asiatica*dose of 500mg/kg bw	$2.20^{b} \pm 0.83$
VI. Provision of benzo (a) pyrene and water extract *C.asiatica* dose of 750 mg / kg bw	$2.60^{b} \pm 1.14$

board and fixation devices, micro pipette (Socorex®, Switzerland), analytical balance (Chyo® Jupiter C3, 100MD), gram scales electrically (PJ, Precisia® Junior, Switzerland), binocular microscope (Olympus®, Japan), camera (Olympus®, Japan).

The water extract of *C. asiatica* leaves was obtained from the Faculty of Pharmacy, Gadjah Mada University. benzo(a) pyrene as carcinogenic material was obtained from SIGMA Chemical Co. USA. Dimethylsulfoxide (DMSO), solvent extract 0.5% CMC Na of Bratacho, Surabaya and distilled water that has a degree of pure reagent were obtained from E. Merck. Formalin was obtained from Bratacho, Surabaya.

Research Procedures

Mice were obtained from the Pusat Pengembangbiakan Hewan Laboratorium Pusvetma Surabaya. The mice were mated and waited until born. The Newborn mice was separated without regard to gender. Four groups were injected intraperitoneally with a solution of benzo(a) pyrene in dimethylsulfoxide (DMSO) on day1st, 8th, 15th post nathal with each dose of 0.2 μmol; 0.4 μmol; and 0.8 μmol.

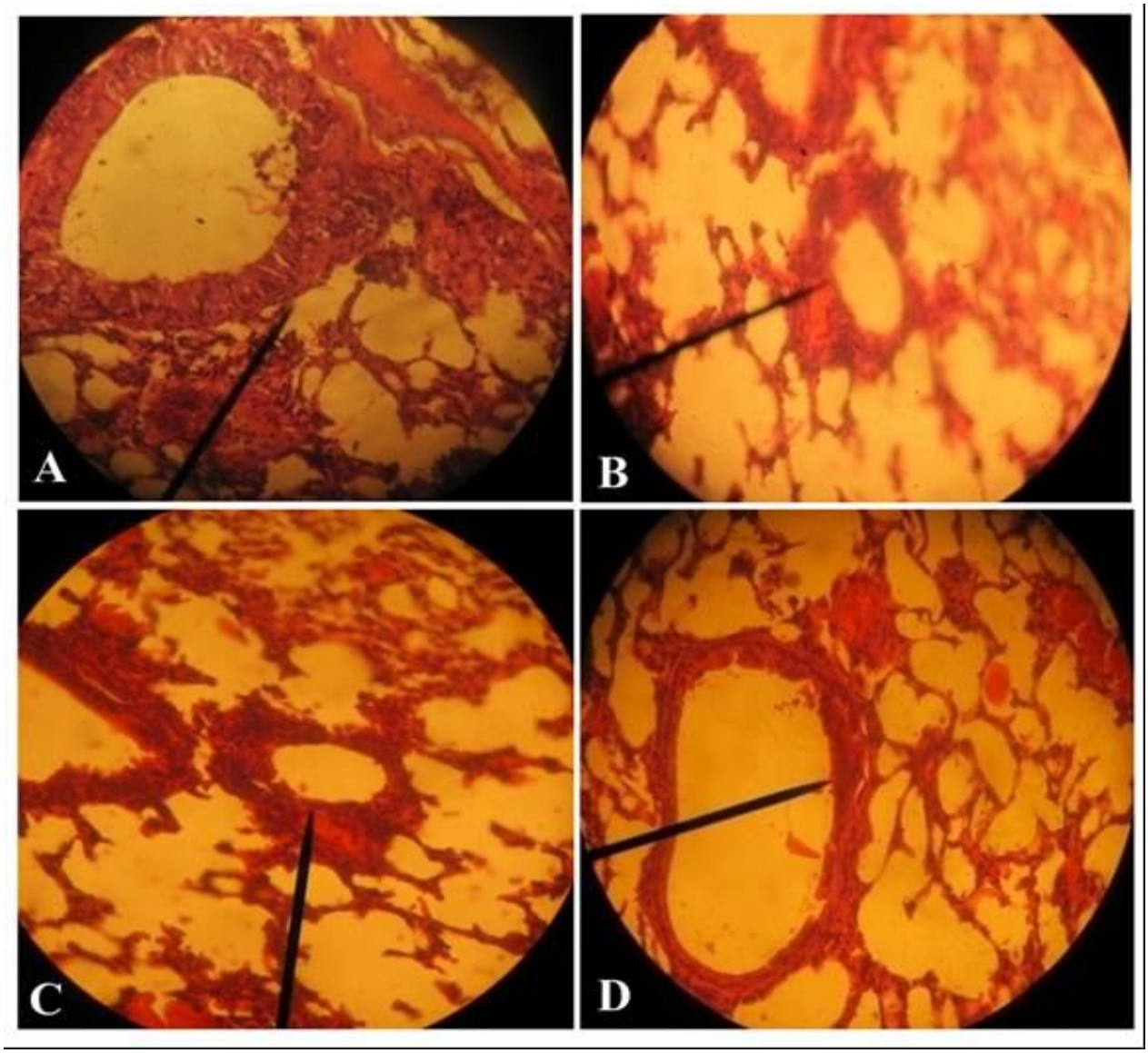

Figure 3. Lung histopathology of mice which given Benzo(a)pyrene and water extract of *C.asiatica*. Microscope with 400x magnification.
Description: The tip arrow was the focus of tumors in alveolar septa. A) Control group B) Tamoxifen group, C) Group of *C. asiatica* Extract with 250mg/kg bw and D) Group of *C. asiatica* Extract with 500mg/kg bw.

The volume of each injection was 20μL. One group was injected DMSO with a dose and the same time as B(a)P as solvent control DMSO. At day 21st, the mice was weaned from its mother mice, then male and female mice housed was separated to avoid a marriage during carcinogenicity test[6].

The Mice that had been injected with B (a) P were divided into six groups: Group I, as a control was only given Benzo (a) pyrene. Group II as a comparison group was given tamoxifen, Group III were given solvent control. Group IV, V and VI were given water extract of *C. asiatica* leaves with variant doses of 250, 500 and 750 mg / kg. Each group consisted of five males mice. The Exposure of *C. asiatica* leaves water extract were twice a week for 8 weeks, starting on day 25 after birth. At the end of treatment (about 4 months), all groups of mice were sacrificed and necropsy performed in order to calculate the number of tumor nodules growing and lung organ preparation for histopathology of focal tumors and hyperplasia observations.

Data Analysis

The number of tumor nodules in each treatment group were counted and compared, then analyzed with ANOVA F-test, if it provided meaningful results, then followed by Duncan's multiple range test. Data were analyzed by using software program SPSS version18.0. Lung histopathology was observed in descriptive qualitative and compared between each treatment group.

RESULTS AND DISCUSSION

The Observations of Total Tumor Lung Nodules

Necropsy when performed on lung organ at the end of treatment for all groups of experimental animals, i.e. when the mice were 16 weeks or four months. The

presence of nodules and number of lung nodules were observed and compared the mean of each treatment group. Lung nodules which formed on the treatment of benzo(a)pyrene was appear white bumps, small and hardens on the surface of the lung, localized not be formed anywhere. Lung tumor nodules would appear more clearly after put in10% buffered formalin (Figure 1 and 2). Furthermore, the presence of lung tumor nodules, then the average of aggregated were counted for each treatment (Table 1).

Based on the analysis of variance F-test, the results of the lung tumor nodules average number show a significant differences ($p<0.05$) on the pattern of water extract of *C.asiatica* leaves. It means that the treatment of water extract *C.asiatica* leaves can inhibit the development of lung tumor nodules.

The differences on mean numbers of lung tumor nodules in each treatment group were analyzed by using Duncan's Multiple RangeTest. The results of the comparison the average number of tumor nodules obtained in the positive control group of benzo(a) pyrene is 5.60±2.07.The mean number is significantly different ($p<0.05$) compared with the group which treated with water extract of *C.asiatica* leaves doses of 500 and 750mg/kg bw. Whereas, when compared to extract with dose of 250mg /kg bw shows no significant difference ($p>0.05$). In the group of mice which only given DMSO and 0.5% CMC Na showed no obtainment of lung tumor nodules.

The smallest meant of Groups of mice which given synthetic drugs for cancer Tamoxifen yield growth in the number of tumor was 2.00±1.58. The results show no significant differences ($p <0.05$) in the group of mice which given water extract of *C.asiatica* doses 500 and 750mg/kg bw equal to 2.20±0.83 and 2.60±1.14. There is an increasing in the number of tumor nodules on the provision of water extract *C.asiatica* dose 750mg/kg bw when compared with dose 500mg/kg bw. So, it means that the optimum dose of *C. asiatica* water extract is 500mg/kg bw for mice induced by benzo(a) pyrene. The higher dose would decrease the potential of anti-cancer. It is predicted that compounds in herbs, such as flavonoids and tannins can trigger the metabolism of enzymes phase II, namely glutathione-s-transferase (GST). The role of GST was to detoxificat the xenobiotic compounds in the body through conjugation with glutathione (GSH). The increasing of GST activity was due to the increasing doses of the extract, then speed up the metabolism process of xenobiotic compounds, including the extract itself, so that the working period and the effect of the organ or tissue will decrease. It was known as auto-metabolism[7].

The Treatment of *C. asiatica* extract with dose 250mg/kg bw could not inhibit the growth of tumor nodules, as seen in the observations result that the mean number of tumor nodules is 4.80±1.64 and shows no significant differences ($p>0.05$) compared with the control group which given Benzo(a)pyrene that equal to5.60±2.07.

Overall it can be said that of 250 and 750mg/kg bw of *C. asiatica* leaves extract can inhibit the growth of lungs tumor nodule sof mice induced byB(a)P. The recent research was stated that the extract of *C.asiatica* increased the phosphorylation of cyclic AMP response element binding protein (CREB) in neuroblastoma cultured cells that expressed beta amyloid 1-42(A beta). Thus was preventing cell proliferation toward malignancy[8].

Microscopic Observations of Pulmonary

The lungs of mice were prepared for histopathological and stained by HE, each treatment were prepared one preparat and then compared descriptively. Histopathological observations which undergone lung tumor nodule formation was due to the initiation by B(a)P covers, the presence of bronchial epithelial hyperplasia, and tumor in bronchial and alveolar septa (Figure 3).

Lung histopathology of mice which induced by B(a)P without *C.asiatica* extract and tamoxifen, shows a hyperplasi of bronchus epithelial and the presence of tumor at alveolar septa and lumen of the bronchi and bronchioles. The picture seems more when compared with group *C.asiatica* extract and tamoxifen administration. The tumor on the septum alveoli became the main focus and caused alveolar septa appear more broken or interrupted, so that between the alveoli became less limitless. The Genetic changes started when there were carcinogenic substances exposure. Polycyclic Aromatic Hydrocarbons general class (PAH), specifically benzo(a)pyrene or B(a)P plays a role in inducing lung cancer. It begun with the biotransformation of B(a)P by cytochrome P450 especially isoforms CYP1A1 be particularly oncogenic compound benzo(a)pyrene diolepoxide (BPDE). These compounds reacted with deoxyguanosine-N^2 (d Guo-N^2), and caused DNA adducts (Figure 1) then became pre-mutagenic event[9].

Microscopic development of carcinogenesis in cells and tissues gave an overview of the more tenuous linkage between cells or tissues as a result of the increasing disconnection of communication signals between cells. The previous research suggested that early stage epithelial neoplastic lesions was maintaining epithelial phenotype, including inter-cell contact-mediated adherens junctions and desmosomes, which also occured in the epithelial to mesenchymal transition (EMT), where EMT p53 mutationis described as control without limiting the activity of p53 when contact between cells was reduced[10].

The existence of interactions between fibroblasts mediated by cadherin hemophilic interaction, focal adhesion molecule mediated cell matrix interactions by integrins. It Important to note that β-catenin was an important signaling molecule. Deregulation of β-catenin, worked as a transcription factor that could trigger p53 activity without DNA damage[11].

The Appearance of tumors was due to the bronchus epithelial cell hyperplasia and cell proliferation that invaded alveolar septa direction. The previous study suggested an exposure of carcinogens substance in the channel of aero digest material, for example tobacco smoke was resulted on changes in the overall which produced locion preneoplastic lesions and lead to differences in the form of multiple primary cancer. It occured in 4-7% incidence of second primary tumors in

patients with primary carcinoma on the head, neck and lung[12].

Post-initiation phase of carcinogenesis, from hyperplasia to carcinoma consisted of chemical treatment target in cancer. There was a possibility that some of the preventive agents could attack the tumorigenesis formation from hyperplasia into carcinoma. Some agents however could be active at a very short distance, particularly on preventing or slowing down hyperplasia, adenoma progression of hyperplasia or adenoma into carcinoma[13].

CONCLUSION

Based on the obtained results, it could be concluded: Treatment water extract of *C.asiatica* leaves in some doses could decrease the number of lung tumor nodules in mice induced by benzo(a)pyrene. The results of mice lung histopathology which induced by benzo(a) pyrene, showed that the water extract of *C.asiatica* leaves in various doses could reduce the formation of a tumor, especially in bronchioles and alveolar septa.

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