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Antitumor Effect of *Phyllanthusniruri* on Irradiated Breast Cancer Tissue in Rat Through Activation of IFNγ and CD95

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ABSTRACT

This study aims to investigate whether the aqueous extract of Phylanthus niruri administered orally contribute to the antitumor effect on irradiated breast cancer in rats.interferron gamma and cluster of differentiation 95 (CD95)had benn knownto have an antitumor effect.An experimental study was carried out in rat with breast cancer induced by the 7.12-Dimethylbenz (a) anthracene (DMBA) and then irradiated (6Gy). The research sample were divided randomly in 3 groups (pretest, treatment and control). Each group had 10 rats. The treatment group was given the plant extract orally (250 mg/kg. b. wt). A significant increase in the number of cells expressing IFNγ($p<0.05$) and a significant increase in the number of cancer cells expressing CD95 ($p<0.05$) in the treatment group were observed. The result indicated that oral administration of Phyllanthus niruri extract increased the antitumor effect on Irradiated breast cancer in rats through activation of IFNγ and CD95.

KEYWORDS : Antitumor effect, IFNγ, CD95, Phyllanthusniruri , and radiation.

The extract of Phyllanthus species has been known to have an antitumor effect (1,2)and induce apoptosis of cancer cells (3,4).Cluster of differentiation 95 (CD95)isa transmembrane protein and after binding to its receptor, can induce apoptosis and play an important role in the regulation of the progression of cancer.(5,6).interferron gamma(IFNγ) is able toacts asan antitumor and it also activate immune cells. IFNγ induces CD95 and its response may result apoptosis(7,8).Radiation therapy damages cancer cells directly by emitting high energy radiation and indirectly by forming free radicals (9).

The aims of this study are to investigate whether the extract of *Phyllanthus niruri* administered orally contributes to the antitumor effect on irradiated breast cancer in rats by increasing the number of cells expressing IFNg and by increasing the number of cells expressing CD95.

MATERIAL AND METHODS

Animals

The study was conducted on healthy female white rats, *Rattusnorwegicus* of *Sprague Dawley* strain, with the body weight of 250 -300 grams obtained from the Animal Breeding Unit of the Integrated Research & Testing Laboratory of Gajah Mada University, Yogyakarta. The rats were acclimatized for 7 days in the animal house andtreated in a well-ventilated polypropylene cages under the standard condition of temperature and humidity. Water and standard pellet diet were provided *ad libitum*. The experiments were conducted after getting an ethical clearance from the Research Ethical Committee of the Veterinary Faculty of Airlangga University, Surabaya.

Experimental design

Thirty irradiated rat with breast cancer were randomly divided into three groups, namely: pretest, treatment, and control groups. Each group had 10 rats. Pretest group was sacrificed at the time before the administration of the plant extract on the treatment group, to prove the existence of cancer cells on breast tissue of rats. Therefore the research design was *randomized separate pretest - posttest control group design*.

Chemicals

The 7.12-Dimethylbenz (a) anthracene (DMBA) (from *Sigma Chemicals Co.,Deisenhofen, Germany)* was dissolved in corn oil at concentration of 4mg/ml.

Breast cancer of rat

DMBA mixed with the corn oil was given at the dosage of 20 mg/kg of rat body weight. The induction was given twice a week for ten times intragastrically through the oro-gastric tube. Assumption on the presence of breast cancer was conducted by the observation and palpation on the nodule at the rat breast during the observation period for 25 weeks after the DMBA induction. To identify the existence of cancer cell, excision of breast tissue of rat was performed and then histologicalpre preparation and microscopic examination were conducted(10). The presence of large nuclei, increased number of mitotic cells and changes in network architecture network, was characteristic of the presence of carcinoma (11).

Irradiation

The rats with nodule on their breasts were treated with a single dose of irradiation of 6Gy (600 rads). Irradiation was performed by using the Teletherapy Cobalt 60 (*Shinva,* made in *People's Republic of China)*. The rats were fixed mounted at special board without any anesthetic application. Irradiation exposure was provided in a field size of 10 x 15 cm^2 at rate of 1,40Gy/min with a distance of 80 cm from the source (12,13).

Phyllanthus niruri extract

Phyllanthus niruri was extracted from the whole parts of the plant in the form of powder (obtained from *Xi'an Biof Biotechnology Co. Ltd, People's Republic of China*) with Certificate of Analysis. The powdered extract was dissolved at a concentration of 10% in double distilled water. Administration of the extract was started on the 6th day after irradiation performed, once per day intragastrically (through the oro-gastric tube) untill the 12th day, at a dose level of 250 mg/kg of the rat body weight (12,13).

Histopathological examination

Thirty days after administration of Phyllanthus niruri extract, all of the rats in the treatment group and control group were sacrified and histological preparation was made from their breast tissues.The examination of IFNg and CD95 was performed by using the immuno histochemistry (IHC) (*Dako, CA,USA*).

Quantitative evaluation

The cells expressing IFNg and the cells expressing CD95 were evaluated by using the light microscope *(Nikon, YS100,Japan*) with 400 times magnification. They were counted separately on 10 fields of view then the average was taken. The histological evaluation was carried out by two persons blindly.

Statistical analysis

The result from IHC was expressed as the mean ± Standard Error Mean (SEM). The data were analyzed using the *Student's t-test*.

RESULTS AND DISCUSSION

This result showed that mean of the number of cell expressing IFNg on the treatment group (12.20 ±4.14) were higher in number compared to the control group (0.60±0.16) or to the pretest group(2.70±0.21) The mean of the number of cell expressing CD95 on the treatment group (7.21±2.10) were also higher in number compared to the control group (4.20±1.31) or to the pretest group (1.10±0.37) (Table-1).

Table-1:
The number of cells expressing IFNg and the number of cells expressing CD95 on Irradiated Breast cancer Tissue in Rats and given the Phyllanthus niruri extract

Group (n=10 of each)	Cell number/field of view	
	IFNg	CD95
Pretest	2.70±0.21	1.10±0.37
Treatment	12.20 ±4.14	7.21±2.10
Control	0.60±0.16	4.20±1.31

Value was expressed as mean +/- SEM. The treatment group was given the Phyllanthus niruri extract orally (250mg/kg b.wt). IFNg and CD95 was performed by immuno histochemistry. Quantitative evaluation of histology was done by two person blindly using the light microscope with the magnification of 400 times, counted separatlye on 10 fileds of view and then the average was taken.

The result of the t-test showed that there was a significant difference of the number of cells expressing IFNg between the pretest group and the treatment group ($p<0.05$) and between the treatment group and the control group ($p<0.05$). Similarly, the result of t-test of the number of cell expressing CD95 showed that there was a significant difference between the pretest group and the treatment group ($p<0.05$) and between the treatment group and the control group ($p<0.05$).

Indirect process of radiation therapy to eliminate cancer is by forming free radicals that induce apoptosis (9). Apoptotic program is regulated by intrinsic signal and extrinsic signal. Regulation by extrinsic signals that rely on the interaction of extracellular ligands with specific transmembrane "death" receptors(14).

CD95 (CD95L or Fas) is a ligand that belongs to the tumor necrosis factor (TNF) family, that bind with its receptor and induces apoptosis (5,6). Ligand-receptor binding in the extrinsic apoptotic signaling , would be facilitated by molecular adapter FADD(Fas associated death domain protein). FADD then recruits pro caspase-8 to form the DISC (Death Encouraging Signalling Complex) and activate caspase-8. Active caspase-8 cleave and activate effector caspase such as caspase-3,-6, and -7, and then direct destroy the cells directly (14).

Interferron (IFN) is a protein that plays a role in communication between cells. This proteins made and released by host cells in response to the presence of viruses, bacteria, parasites or tumor cells. Cellular effects of IIFNg are up-regulation of pathogen recognition, inhibition of cellular proliferation, immuno modulation, and effects on apoptosis. In addition, it is also integrate signaling and response with other cytokines and pathogen-associated molecular patterns(15).

besides have an antiinflamation and anti tumor effects, IFNg can induces CD95 (FAS). Research on Trypanosomacruzi infection in mice , administration of IFN-γ is able to modulate the induction of apoptosis by two independent and different mechanisms, there is induction of NO production and of Fas and Fas-L expression (8).IFN-γ is also induces sensitivity of endothelium to CD95L-mediated apoptosis, and that this response may result from increased expression of CD95 and/or pro-caspase-8 (7).

The mechanism of antitumor effect of the extract of *Phyllanthus species* was conducted by the decrease of cyclooxygenase-2 (COX2) and interleukin-8 (IL-8), anti-apoptosis, and also by the induction of tumor necrosis factor-α (TNF-α), pro-apoptosis (4).Another research explained that this mechanism was due to the decrease of activities of Bcl2, anti-apoptosis, and the increase of caspase-3, the apoptosis executor (3). Administration of the extract of *Phyllanthus niruri* on DMBA induced breast cancer in irradiated rats in this study can increase the number of cell expressing IFNγ and CD95. The result indicate the presence of another mechanism of antitumor effect of this plant extrct in inducing apoptosis it is mean an antitumor effects.

Phyllanthus niruri had some chemical components and each had a different effect. Different classes of organic compound with various medical interest have been reported, the major being the lignans, tannins, polyphenols, alkaloids, flavonoids, terpenoids and steroids (16,17). The type of organic compound that induce expression of IFNγ and CD 95 was not determined in this study. The immune cell types that express IFNg or the immune cell types that express CD95 is also not determined.This was the weakness of the study. The use of different rats on the pretest and treatment group was also must be a concern, although the study design had resolve the problem.

The present study opens a perspective for the use of the extract of *Phyllanthus niruri* in radiation therapy. The extract might serve as a radiosensitizer since it is able to acts as an antitumor effect and inducingapoptosis by increasing the number of cell expressing IFNg and CD95. Further research is still needed.

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