



## Protective effect and antitumor effect of Phyllanthus niruri extract on DMBA-induced breast cancer in irradiated rats.

### KEYWORDS

Apoptosis, Heat Shock Protein 70, Phyllanthus niruri, and radiation

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### ABSTRACT

*This study aims to investigate whether the aqueous extract of Phyllanthus niruri administered orally not only contribute to the antitumor effect but also provide a protective effect on breast cancer in irradiated rats. An experimental study was carried out on 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 Heat Shock Protein 70 ( $p < 0.05$ ) and a significant increase in the number of cancer cells undergoing apoptosis ( $p < 0.05$ ) in the treatment group were observed. The result indicated that oral administration of Phyllanthus niruri extract not only increased the antitumor effect but also provided a protective effect on DMBA induced breast cancer in irradiated rats.*

### INTRODUCTION

Radiation therapy damages cancer cells directly by emitting high energy radiation and indirectly by forming free radicals inducing apoptosis. The free radicals can also damage the healthy nearby cells and cause radiation side effect (Woodward, 2008).

The extract of Phyllanthus has been known to have an antitumor effect (Rajkapoor et al, 2007; Islam et al, 2008) and induce apoptosis of cancer cells (Huang et al, 2003; Sureban et al 2006, ). Paradoxically, this plant extract also has a protective effect on cells by inducing antioxidants (Karuma et al, 2009) and giving a radioprotective effect (Kumar et al, 2004; Kumar et al, 2007).

Heat Shock Protein 70 (HSP70) is able to provide protection to the immune system (Jaqcquier et al 1994), protection against stress which can induce apoptosis (Moser et al, 2000), and protection against heat stress (Fu & Tupling, 2009).

The aims of this study are to investigate whether the extract of Phyllanthus niruri administered orally contributes to the antitumor effect on breast cancer irradiation in rats by increasing the number of cancer cells undergoing apoptosis of cancer cells and simultaneously the plant extract also has a protective effect against irradiation by increasing the number of cells expressing HSP70.

### MATERIAL AND METHODS

#### Animals

The study was conducted on healthy female white rats, Rattus norvegicus 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 and treated 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 divid-

ed 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 histological preparations and microscopic examination were conducted. The presence of large nuclei, increased number of mitotic cells and changes in network architecture network, was characteristic of the presence of carcinoma (Kubatka et al, 2002).

#### 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<sup>2</sup> at rate of 1,40 Gy/min with a distance of 80 cm from the source (Kumar et al, 2004; Kumar et al, 2007).

#### 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) until the 12th day, at a dose level of 250 mg/kg

of the rat body weight (Kumar et al, 2004; Kumar et al, 2007).

### Histopathological examination

Thirty days after administration of Phyllanthus niruri extract, all of the rats in treatment group and control group were sacrificed, and histological preparation was made from their breast tissues.

Apoptosis examination was performed by using Terminal deoxynucleotidyl transferase dUTP nick end labeling (Tunel) assay (Dako, CA, USA) while the examination of HSP 70 was performed by using the immunohistochemistry (IHC) (Dako, CA,USA).

### Quantitative evaluation

The cancer cells undergoing apoptosis and the cells expressing HSP70 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 Tunel assay and IHC was expressed as the mean  $\pm$  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 HSP70 on the treatment group ( $5.50 \pm 0.45$ ) were higher in number compared to the control group ( $1.10 \pm 0.23$ ) or to the pretest group ( $3.20 \pm 0.24$ ). The mean of the number of cancer cells undergoing apoptosis on the treatment group ( $8.30 \pm 0.42$ ) were also higher in number compared to the control group ( $5.50 \pm 0.26$ ) or to the pretest group ( $1.10 \pm 0.37$ ) (Table-1).

The result of the t-test showed that there was a significant difference of the number of cells expressing HSP70 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 cancer cells undergoing of apoptosis 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 ( $p < 0.05$ ).

The results of this study indicated that administration of the extract of Phyllanthus niruri on DMBA induced breast cancer in irradiated rats can increase the apoptosis of cancer cells and can increase the number of cells expressing HSP70.

Phyllanthus niruri has been known to have an effect of immunomodulator (Choudari et al, 2011; Zalizar, 2011). The plant extract of Phyllanthus can improve antioxidant status and reduce the risk of oxidative stress (Karuma et al, 2009). In this study, the plant extract was able to increase the activity of disturbed cells due to irradiation side effect by increasing the number of the cells expressing HSP70.

The mechanism of antitumor effect of the extract of Phyllanthus 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- $\alpha$  (TNF- $\alpha$ ), pro-apoptosis (Sureban et al, 2006). 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 (Huang et al, 2003). An increase in the number of cancer cells undergoing apoptosis in this study was in accordance with the previous research.

Research on the effect of administration of the extract of Phyllanthus on irradiated mice indicated that there was an increase in the total white blood cells, an increase of cellularity of bone marrow, and a decrease of lipid peroxidation

levels in serum and liver, which was the radioprotective effect (Kumar, 2004). Other studies on the effect of administration of this plant extract had reported the presence of the decreased in intestinal cell damage because of side effect of radiation on mice. This extract also protected the clastogenic effect because of radiation, as seen from decreasing number of micronuclei and there was also a decreased on the percentage of chromosomal aberration (Kumar, 2007). The increase in cells expressing HSP70 as given Phyllanthus niruri provide protection to proteins against irradiation and allows the protein to maintain its function. It was a radioprotective effect of the plant extract.

In this study, the antitumor effect and the protective effect on breast cancer in irradiated rats seemed paradoxical and occurred simultaneously. This was possible because 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 (Calixto et al, 1998; Bagalkotkar et al, 2006).

The weakness of this research was it did not determined the immune cell types that express HSP70. The radioprotective effect of Phyllanthus niruri on breast cancer in irradiated rats should also be assessed whether it will reduce the potential for antitumor effect of this plant extract. 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 increases the apoptosis of irradiated breast cancer cells. Simultaneously the extract might serve as a radioprotector since it protects protein from the irradiation side effect.

**Table-1: Cancer cells undergoing apoptosis and cells expressing HSP70 on DMBA-induced breast cancer in irradiated rats.**

| Group<br>(n=10 of each) | Cell number/field of view |                 |
|-------------------------|---------------------------|-----------------|
|                         | HSP70                     | Apoptosis       |
| Pretest                 | 3.20 $\pm$ 0.24           | 1.10 $\pm$ 0.37 |
| Treatment               | 5.50 $\pm$ 0.45           | 8.30 $\pm$ 0.42 |
| Control                 | 1.10 $\pm$ 0.23           | 5.50 $\pm$ 0.26 |

Value was expressed as mean  $\pm$  SEM.

The treatment group was given the Phyllanthus niruri extract orally (250 mg/kg. b. wt).

Apoptosis examination was performed with Tunel assay and HSP 70 with immunohistochemistry.

Quantitative evaluation of histology was done by two persons blindly using the light microscope with the magnification of 400 times, counted separately on 10 fields of view, and then the average was taken.

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