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CYTOTOXICITY EFFECT OF *Curcuma aeruginosa* EXTRACT ON FIBROBLAST WITH MTT ASSAY METHOD

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ABSTRACT

The aim of this study was to find cytotoxicity effect of Curcuma aeruginosa extract on fibroblast with MTT assay method. This study used experimental laboratory with post test only control group design. Fibroblast cell lines culture divided into ten groups with four replication in each group. The positif control group was the group contained fibroblas cells in RPMI media. The negatif control group was the group contained media without cells. There were eight treatment groups given Curcuma aeruginosa extract in different concentration (1 ppm, 2 ppm, 2.5 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm). After exposure of the extract, the cells were incubated for twenty hours. The MTT reagen was given on the cells and then was incubated for four hours. After that the optic density was read with microplate reader in 630 wavelength. The result showed that the percentage of viable cells was more fifty percent in treatments groups. The Anova analysis showed that there was significantly decrease ($p<0,05$) of percentage of viable cell between positif control group and treatment groups. The conclusion was the Curcuma aeruginosa extract did not show cytotoxicity effect on fibroblast cells but the percentage of viable cell was decrease significantly in treatment groups.

Keywords: *Curcuma aeruginosa extract, fibroblast, cytotoxicity, MTT assay.*

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INTRODUCTION

Temu Ireng (*Curcuma aeruginosa*) has been known quite widely for the treatment of diseases worms, cleaning the blood in women after childbirth, appetite stimulant and can be as foreign drugs for skin diseases. Temu Ireng containing starch, saponins, flavonoida, polyphenols and essential oil which is the active substance as a medicine for worms. Inside there are volatile oil fraction and the fraction of terpinen-sabinen 4-01. The second fraction containing a substance suspected of being active against worms and worm eggs (Syamsuhidayat & Hutapea 1991, Taroeno 1990). Intersection of research conducted by Hestianah Ireng (1996) in laying hens is in helminth infection of Ascaridia galli, showed a decrease in the production of worm eggs per gram of feces, but the side effects of diarrhea and symptoms of liver damage microscopically in the laying hens.

The use of traditional medicine Intersection Ireng as not entirely safe, therefore to use as a medicinal Ireng meeting must be safe and acceptable by the body or does not harm patients who take them. Medicinal plants contain various chemical constituents with the amount of the chemical content varies. Total chemical content varies as determined by many factors such as age of plants, places to grow, and seeds are used. These circumstances may cause the effects vary. Besides varying effects may be caused by differences in the dosage form is used. Various extracts produced by different types of solvent can have different effects (Klaassen & Watkins 1999).

One result of gastrointestinal infection is the destruction of worms in the intestinal wall due to penetration of the worm (Grimes et al. 1991). Certain types of worms will be attached to the intestinal mucosa causes chronic bleeding and damage to the intestinal mucosa (Pohan 1996). In the lamina propria mucosa, small intestine, containing fibroblast populations that often experience replication. Fibroblasts are the main cells in the connective tissue, capable of producing the material between cells. Composed of epithelial cells tightly arranged, avaskuler, little extracellular matrix. Epithelial cells have a limited life skills, often have flaking, cell death, and the experience of differentiation. This process causes the loss of the ability of cells to divide. Intestinal epithelium is a layer of cylindrical epithelium, with basal lamina beneath. Presence of fibroblast cells in intestinal mucosal lamina propria, very important role in the repair of epithelial damage (Fawcett & Jensh 2002, Ross et al. 2003).

Fibroblast cells that is often used in studies in vitro, due to fibroblast cells is easier and cheaper than the

epithelial cells to be grown. Inside the small intestine mucosa, sloughing of epithelial cells often have more stable while the fibroblast cells in the lamina propria. Source of fibroblast cells can be acquired from primary culture or using cell lines. There are two types of fibroblast cells that is usually used in cell culture lines, namely L-29 cells derived from rat lung fibroblasts and BHK-21 cells derived from hamster kidney fibroblasts. BHK-21 cells but is more widely used to test the cytotoxicity of materials and medicines. This is caused by Baby Hamster Kidney fibroblast cells (BHK-21) is the best culture material derived from embryonic tissue cells so easy to grow and easy to perform repeated subculture (Freshney 1987, Rantam 2007).

According to Siregar and Hadijono (2000), one valuation method in vitro cytotoxicity of a material is by enzymatic test using MTT reagent, namely purple soluble molecules that can be used to assess the cellular enzymatic activity, based on the ability of living cells to reduce the salt MTT (3 - (4.5-dimethylthiazol-2-Joel) - 2.5-di-phenyl-tetrazolium bromide). Based on the background that has been described it is necessary to extract cytotoxicity test Ireng Intersection of fibroblast cells that is useful to know the concentration is safe for the body and causes no damage to the network.

MATERIALS AND METHODS

Type of research is experimental laboratory with the study design-only The Post-Test Control Group Design In this study, the concentration used was 1 ppm, 2 ppm, 2.5 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm. Rhizome extract preparation process Intersection Ireng (Curcuma aeruginosa) is conducted at the Laboratory of Natural Products Sciences, Faculty of Pharmacy, Airlangga University Surabaya.

Rhizome extract preparation procedure Ireng meeting is to take 500 mg Intersection net Ireng already sliced and in the wind-wind to dry and then is pulverized, soaked powder Ireng meeting in 1500 cc 96% ethanol for 24 hours, filter and filter paper soaked with placed on Erlenmeyer tube connected to a vacuum device to suck the liquid, making the screening process at least five times, then process the filtrate with rotaevaporator to get ethanol-free extracts, extracts Intersection Ireng take as much as 20 mg dissolved in 1 cc of DMSO. To create a homogeneous solution, dissolved by means of ultrasonic way Ultrasoner (Branson 3510) for 5 minutes. Further meetings will be obtained extract solution Ireng 20 000 ppm concentration (called mother liquor). In every 1 µl of this mother liquor containing 20 µg extract Ireng Intersection. From the mother liquor was taken 5 µl then added 100 µl of media solutions, so that each 1 µl containing 1 µg extract Ireng Intersection. For concentration of 25 ppm treatment group, used 5 µl plus 195 µl of media. Furthermore, to obtain the concentration of the extract Intersection Ireng below 25 ppm, done several times dilution in the same way.

The way work is done by sterilizing all tools and materials to the autoclave temperature is 121oC for 15 minutes, BHK-21 cells were cultured in a cell-line implanted in roux bottles. Once confluent, cultures were harvested using versene trypsine solution. The crop is planted in the memorial institute medium rosewellpark-1640 (RPMI-1640) containing 10% fetal bovine serum albumin were incubated for 24 hours the temperature of 37^{o}C. Then the cell was moved in small bottles (roux) and made with a density of 2 x 105 cells / ml. Tues cultured in each well mikroplat 96-wells until confluent. Each shaft contains cells and RPMI medium with a density of 2 x 105 cells / ml. Each treatment group met Ireng plus extract concentration of 1 ppm, 2 ppm, 2.5 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm. For positive konrol groups and the media containing cells, whereas the negative control group containing only media without cells. Then the plate wells were incubated for 20 hours at a temperature of 37^{o}C. After that, each pitting added reagent MTT 5 mg / ml in PBS as much as 20 µl for each shaft, incubated again for 4 hours at a temperature of 37^{o}C. Furthermore, each pitting plus 50 µl dimethyl sulfoksid (DMSO) (which serves to stop the process of absorption of MTT by the mitochondria). Plate wells were incubated again for 5 minutes at a temperature of 37^{o}C. Then read on spectrophotometer plate wells with a wavelength of 630 nm. The result obtained is expressed in optical density / absorbent. Large absorbent every pitting shows the number of living cells in culture media.

Once read in by readers mikroplate, then optical density data obtained for each shaft, both in the control group and treatment group. Data were analyzed by descriptive statistics, normality test data, test of homogeneity of variance, one-way ANOVA, multiple comparison test.

RESULTS

Data on the optical density of each group can be seen in the table 1. From table 1 can be seen that the positive control group, the highest optical density value of 0.10800, while the lower density values contained in the negative control group amounted to 0.05100. In the group treated with various concentrations showed the highest density value on the concentration of 1 ppm group, amounting to 0.09300, and the lowest value at concentrations of 2.5 ppm group. From one-way ANOVA test showed $p = .000$ ($p < 0.05$), which means

that there are significant differences in density values in each group. Test test followed by multiple comparisons, and found that there are significant differences between treatment groups with the negative control group and positive control group.

Table 1 Values of Optical Density of the Study Group

Group	Average	SD	Minimum	Maximum
Control -	0.05100	0.000816	0.050	0.052
Control +	0.10800	0.010863	0.095	0.120
1 ppm	0.09300	0.004243	0.089	0.099
2 ppm	0.09025	0.005252	0.083	0.095
2.5 ppm	0.07700	0.009416	0.071	0.091
5 ppm	0.08125	0.007632	0.072	0.090
10 ppm	0.07875	0.006500	0.073	0.088
15 ppm	0.07925	0.006602	0.072	0.088
20 ppm	0.07850	0.003416	0.075	0.083
25 ppm	0.07875	0.011843	0.071	0.096

Table 2 Mean Differences of Optical Density on the Study Group

Group	C -	C +	1 ppm	2 ppm	2.5 ppm	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm
C -		0.0570*	0.0420*	0.0392*	0.0260*	0.0303*	0.0278*	0.0283*	0.0275*	0.0278*
C +			0.0150*	0.0178*	0.0310*	0.0268*	0.0293*	0.0288*	0.0295*	0.0293*
1 ppm				0.0028	0.0160*	0.0118*	0.0143*	0.0138*	0.0145*	0.0143*
2 ppm					0.0133*	0.0090	0.0115*	0.0110*	0.0118*	0.0115*
2.5 ppm						0.0043	0.0018	0.0023	0.0015	0.0018
5 ppm							0.0025	0.002	0.0028	0.0025
10 ppm								0.0005	0.0003	0.0000
15 ppm									0.0008	0.0005
20 ppm										0.0003
25 ppm										

Note: * = no significant difference ($p < 0.05$)

From the table above, in the treatment group, can be seen at a concentration of 2 ppm, there were significant differences by treatment group concentration of 2.5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm. But from 2.5 ppm treatment group did not give significant differences with higher concentrations (5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm).

The percentage of live cells based on optical density can be seen in the table below:

Table 3 Percentage of live cells and dead cells

Group	Presentation of life cell (%)	Presentation of dead cell (%)
Control -	100	0
Control +	0	0
1 ppm	90.57	9.43
2 ppm	88.84	11.16
2.5 ppm	80.50	19.50
5 ppm	83.17	16.83
10 ppm	81.60	18.40
15 ppm	81.92	18.08
20 ppm	81.45	18.55
25 ppm	81.60	18.40

In the table above, the percentage of living cells in the positive control group was 100%, whereas the negative control group was 0%. In the extract treated groups meet Ireng various concentrations, it appears that the treatment group with the lowest concentration (1 ppm) percentage of live cells decreased to 90.57%, and the lowest percentage at 2.5 ppm group that is equal to 80.50%. In the treatment group percentage of dead cells at the highest concentrations of 2.5 ppm and the lowest in group 1 ppm.

DISCUSSION

Lesion presence on cells caused the changes in these cells. One of the substances that cause a lesion in the cell is a chemical (chemical agents). Cells that are stressed or there fisioligis pathological stimuli that cause cell adaptation. When the cells exceeded the ability of adaptation will be formed cell responses against the occurrence of lesion response of reversible or irreversible. Response causes irreversible cell death, either through the process of necrosis or apoptosis (Kumar V et al. 2003).

Results hydrodistilasi Intersection Ireng rhizome (Curcuma aeruginosa), showed a high content of

compounds monoterpenoids of 59.26% (Srivastava et al. 2006). Inside the compound there monoterpenes alcohol content. The main monoterpenoids contained in Curcuma aeruginosa are cineol and camphor compounds (Grayson 1998). The compound 1,8-cineole and camphor can reduce cell division, result in changes in organelle structure and membrane rupture core and membrane organelles (Muller 1965 cit. Purcaro 2007). Mitochondria are cell organelles that have a membrane. At the membrane, precisely at the cristae of mitochondria, there are proteins that play a role in oxidation reactions of the respiratory chain, ATP synthesis, and regulates the transport of metabolites in and out of the matrix. Enzymes for respiration chain attached to the inner surface with its head stuck up into the matrix. These enzymes cause oxidative phosphorylation to produce ATP (Ross et al. 2003). Kumar et al. (2003) mentions that the decrease in ATP would disrupt the balance of cellular osmolarity. The absence of ATP synthesis through oxidative phosphorylation in mitochondria causes rapid loss of homeostasis path.

Telli et al. (1999) states that a substance toxic to say if the percentage of living cells after exposure to such materials is less than 50% or more dead cells from 50%. In this study, the percentage of fibroblast cells die less than 50% after administration of the extract Intersection Ireng various concentrations, so that it can be said until the concentration of the extract Intersection Ireng 25 ppm, did not cause toxicity in fibroblast cells.
Based on table 2 and table 3 above, can be seen in all treatment groups the percentage of dead cells increased significantly as compared to positive control group. Increasing the percentage of dead cells was significantly different with increasing concentration of extract up to 2.5 ppm. Meanwhile, at a concentration of more than 2.5 ppm are not significant differences in the percentage of cell death.

Increasing the percentage of dead cells was significant, indicating that although the extract Intersection Ireng to 25 ppm dose did not cause toxicity on cells, but showed potential to reduce cell survival. In this research, cell survival can be seen from the ability of fibroblast cells to reduce MTT salt and produce a purple formazan product. This reaction occurs with the enzyme mitochondrial dehydrogenase in living fibroblast cells. The higher the density of formazan formed values indicate increasing numbers of living cells.

Due to the extract Intersection Ireng cineol and camphor-containing membranes causing damage to mitochondria that contain enzymes for respiration. At concentrations of 1 ppm, 2 pp, 2.5 ppm and 5 ppm was found to increase significantly the percentage of dead cells. This indicates increased concentration affects the damage to the organelle membrane.

CONCLUSION

Increasing the number of dead cells that were significant in this study due to the high content of monoterpenes (1,8-cineol and camphor) on the Intersection Ireng. Both these substances cause damage to the membrane of organelles including mitochondria, inner mitochondrial membrane dimanapada there are enzymes for respiration chain. Consequently after the administration of the extract Intersection Ireng there was decrease in the percentage of living cells, indicated by the inability of cells to reduce MTT salt into formazan.

Intersection Ireng extract of fibroblast cells by MTT essay method, at concentration of 1 ppm to 25 ppm showed no toxicity, judging from the percentage of dead cells after treatment is less than 50%. But shows the percentage of dead cells increased significantly between treatment groups with the positive control group.

REFERENCES

Fawcett, DW, Jensh, R P. 2002. Bloom & Fawcett's Concise Histology. 2 nd ed, Oxford University Press, New York, pp. 1-2, 6-7.

Fresney R I. 1987. Culture of Animals Cells (A Manual of Technique). 2 nd ed. New York. Liss. Inc. Pp: 7-12

Grayson, DH, 2000. Monoterpenoids. Natural Product Reports, Nomor 17, 441-446

Grimes, DE, Richard, MG & Mark Hamelink. 1991. Infectious Diseases. St. Louis, Mosby-Year Book, p 96

Hestianah EP, 1996. Pengaruh Rimpang Temu Ireng (Curcuma aeruginosa) terhadap Gambaran Histopatologi Hati dan Usus Halus Ayam yang Diinfeksi Cacing Ascaridia galli. Thesis ,UNAIR, Surabaya

Klaassen, Curtis D dan John B. Watkins III. 1999. Casarett & Doull's Toxicology The Basic Science of Poisons. 5th ed, New york, Mc Graw Hill Companies. Inc, p 695

Kumar, V, Cotran, RS, Robbins, SL, 2003. Robbins Basic Pathology. 7th ed, Saunders, Philadelphia, pp. 4-6

Pohan, Herdiman T. 1996. Penyakit Cacing yang Ditularkan Melalui Tanah. Dalam Sjaifoellah Noer (Ed.) Buku Ajar Ilmu Penyakit Dalam Jilid I, Edisi Ketiga, Jakarta, Balai Penerbit FKUI, hal. 517

Purcaro,R, 2007. Isolation of secondary metabolits from plants and their use as lead compounds for the synthesis of biologically active products. Dottorato di ricerca, Universita' Degli Studi di Napoli Federico II

Rantam, F A, 2007. Tissue Culture. Diktat Pelatihan Metodologi Laboratorium Tissue Culture, Tropical Disease Center, UNAIR, Surabaya.

Ross, MH, Kaye, G I, Pawlina, W. 2003. Histology; A Text and Atlas, 4th ed, Lippincott, Williams and Wilkins, Baltimore, Philadelphia, pp.42-45

Siregar F dan Hadijono BS . 2000. Uji Sitotoksisitas dengan Esei MTT. Jurnal Kedokteran Gigi UI. Vol : 7. Hlm : 28-32.

Srivastava S, Chitranshi N, Srivastava S, Dan M, Rawat AKS and Pushpangadan P, 2006. Pharmacognostic Evaluation of Curcuma aeruginosa Roxb. Natural Prodact Sciences 12 (3) : 162-165.

Syamsuhidayat SS, Hutapea JR. 1991. Inventaris Tanaman Obat Indonesia I. Departemen Kesehatan RI. Badan Pebelitian dan Pengembangan Kesehatan

Taroeno, 1990. Isolasi dan Identifikasi Fraksi aktif Antelmintik dari Rimpang Zingiber purpureum Roxb., Disertasi UNAIR, Surabaya

Telli C, Serper A, Dogan A L, Guc D.1999. Evaluation of The Cytotoxicity of Calcium Phosphate Root Canal Sealers by MTT Assay. J Endodon. Vol 25. p: 811.