

## Cytotoxicity of *Jatropha curcas* (Euphorbiaceae) latex on fibroblast by MTT assay

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### Abstract

The latex of *Jatropha curcas* (Euphorbiaceae) had been used as traditional plant medicine among others to cure toothache. Despite its long time use, not many scientific research on this latex were reported. The aim of this study was to evaluate its cytotoxicity on cell culture. Latex was lyophilized and stored at  $-20^{\circ}\text{C}$  for standardization. Fresh latex was around 15% freeze-dried latex. Fibroblast L929 cell line were exposed to 37-10.000  $\mu\text{g/ml}$  latex in medium for 1, 2, and 3 days and the cytotoxicity was evaluated by MTT assay. The result showed that the number of cells was half of control at concentration of 625  $\mu\text{g/ml}$  and no viable cells were found at concentration of 2500  $\mu\text{g/ml}$  freeze dried latex. Lower concentration of latex was needed to yield similar effect to human gingival fibroblast primary cells. After 2 days the number of gingival fibroblast cells was nearly half of that of control at 150  $\mu\text{g/ml}$  latex solutions. It is concluded, that *J. curcas* latex was cytotoxic to Fib L929 and gingival fibroblast cells.

### Abstrak

Getah *Jatropha curcas* (getah jarak, Euphorbiaceae) telah digunakan sebagai obat tradisional antara lain untuk menanggulangi nyeri pulpa gigi. Namun belum banyak penelitian ilmiah dilaporkan. Tujuan penelitian ini adalah untuk mengetahui sifat sitotoksitas getah *J. curcas* pada kultur sel. Untuk standarisasi, getah dibeku-keringkan dan disimpan pada  $-20^{\circ}\text{C}$ . Konsentrasi getah segar adalah sekitar 15% getah beku-kering. Sel Fib L929 dipapar dengan getah 37-10.000  $\mu\text{g/ml}$  medium kultur selama 1, 2, dan 3 hari. Kemudian sifat sitotoksitas getah dievaluasi dengan assay MTT. Hasil menunjukkan bahwa absorbansi sel Fib L929 menjadi setengah dari absorbansi kelompok kontrol pada 625  $\mu\text{g/ml}$ , dan tidak ada sel yang hidup pada konsentrasi getah 2500  $\mu\text{g/ml}$ . Efek yang sama terhadap fibroblast gingiva manusia ditemukan pada kadar getah yang lebih rendah. Sesudah 2 hari, jumlah sel fibroblast gingiva manusia mendekati setengah jumlah sel kontrol pada larutan getah 150  $\mu\text{g/ml}$ . Kesimpulan adalah getah *J. curcas* bersifat sitotoksik terhadap sel Fib L929 dan fibroblast gingiva manusia.

**Keywords:** *Jatropha curcas* latex, cytotoxicity, MTT assay, fibroblast.

*Jatropha curcas*, an Euphorbiaceae, is a shrub or tree found in Indonesia and other tropical areas. Its local names are balacai (Sulawesi), nawaih nawas (Sumatera Barat), jarak (Jawa Barat), jarak pagar and many others. Latex of *Jatropha curcas* contains tannins, saponin, wax, and resin.<sup>1,2</sup> From the latex were isolated a proteolytic enzyme called curcain<sup>3</sup> and a cyclic octapeptide named curcacyclin A which inhibits the classical pathway of human complement and proliferation of human T-cells.<sup>4</sup> Latex of *J. curcas* showed an antibacterial activity against *Staphylococcus aureus*<sup>5</sup> and a coagulating activity in *in vitro* test.<sup>6</sup> In tropical Africa and Southeast Asia the latex is used as a hemostatic, wound dressing, and is said to be

effective in treating scabies, eczema, and ringworm. Furthermore, it is used as mouth rinse to treat bleeding gums, to touch a baby's inflamed tongue and to cure toothache.<sup>1, 2, 7, 8, 9</sup>

MTT is a microplate assay requiring no cell transfers. This method was adapted for measuring proliferation and cytotoxicity in sensitive, rapid, and semiautomatic manner without radioactive isotope.<sup>10</sup> The MTT assay is based upon the selective ability of living cells to reduce the yellow soluble salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a purple-blue insoluble formazan precipitate.<sup>11</sup> Reduction of tetrazolium salts is intracellular and predominantly involves NAD(P)H-dependent enzyme of endoplasmic reticulum.<sup>12</sup> The number of viable cells is therefore measurable as the concentration of the solubilized MTT reaction product measured in a spectrophotometer.

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The solubility of MTT-reduced formazan product was poor in acid isopropyl alcohol (as used in the method of Mosmann)<sup>11</sup> while better absorption characteristics were observed with mineral oil and DMSO as solvents.<sup>13</sup> The amount of formazan product generated and then measured after solubilization in DMSO is proportional to cell number, although absolute absorbance for a given cell number varies between cell lines.<sup>13</sup>

Despite the long time use of *J. curcas* as a plant medicine among other to cure toothache, not many scientific researches were conducted. After the latex was put in direct contact with the dental pulp, the pain would disappear. The purpose of the study was to explore the mechanism of pain relief and one possibility is that the latex is cytotoxic. In this study, cytotoxicity of latex of *J. curcas* was evaluated by MTT assay on Fib L929 and human gingival fibroblast primary culture.

## METHODS

RPMI 1640 (Sigma), Penicillin-streptomycin (Sigma), MTT (Sigma), fetal bovine serum (FBS, Gibco), Fungizone (Gibco), Hepes (Gibco) and DMSO were used in this study. Fibroblast L929 cell line and human gingival fibroblast were kindly donated by Moekti G., Research Institute for Veterinary Sciences, Bogor, and Leyhausen G., Hannover, respectively. To standardize the sample, latex of *Jatropha curcas* was obtained from *J. curcas* trees grown in Research Institute for Plant Medicine and Spices, Bogor. Subsequently, the latex were lyophilized 50 hours and stored at  $-20^{\circ}\text{C}$ . We found that fresh latex was equivalent with 15% solution of freeze-dried latex.

## Cell culture

Fibroblast L929 and human gingival fibroblast were cultured as monolayers in RPMI 1640 medium and DMEM respectively, supplemented with 10% (v/v) fetal bovine serum (FBS), 25 mM Hepes, 100.000 IU/L penicillin, 100 mg/L streptomycin, and 2,5 mg/L fungizone. Growing cultures were maintained in 25 cm<sup>2</sup> culture flasks at 37<sup>o</sup>C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Medium was changed 2 times a week or when there was a pH decrease shown by colored changes in the medium. At confluency, they were harvested by trypsination and counted by trypan blue staining.

## MTT assay

Into each well of monolayer fibroblast in 96-wells microplate, 20  $\mu\text{l}$  of 5 mg/ml MTT, was added and incubated at 37<sup>o</sup>C for a further 4 hours. Medium was then aspirated and 50  $\mu\text{l}$  DMSO were added into each well. After 15 minutes of incubation, the absorbance (OD) were determined by ELISA microplate reader (Titertek Multiskan MCC) at wavelength 540 nm subtracted by 690 nm.

## Optimization

To evaluate the cytotoxicity of latex on cell culture, certain amount of latex was added into the medium which was supplemented with FBS. In our work we found precipitation formed in medium when added with latex. The higher the FBS and latex concentration, the more precipitation formed. Therefore, optimization of FBS and latex concentrations used in experiment should be done. The amount of FBS in medium assayed was 0%, 5% and 10%, while latex concentration assayed was from 150.000  $\mu\text{g/ml}$  (15%) and diluted by half until 37.5  $\mu\text{g/ml}$  medium (0.003%).

We also found that diluted latex in medium had its own optical density (OD). At 37.5  $\mu\text{g/ml}$  to 150.000  $\mu\text{g/ml}$  the OD at 540 nm ranged from 0.080 to 1.288 (figure 1). Consequently, the cytotoxicity data were obtained from the OD of cells after exposure to the diluted latex subtracted by the OD of latex in medium with the same concentration as those used for cells.

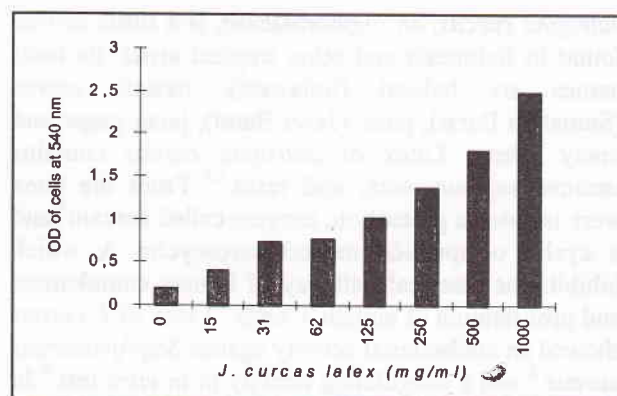


Figure 1. OD at 540 nm of several concentration of *J. curcas* latex in medium. *J. curcas* solution has its own absorbance

## Cytotoxicity test of latex of *Jatropha curcas*

One hundred and fifty microlitre of 40.000 cells/ml was seeded into well of 96-well microplate. After

confluency, the medium was aspirated and changed with a new medium added by several concentrations of latex supplemented by FBS and antibiotics. Same concentrations of latex in medium (without cells) were also put into other wells and treated as those wells with cells. Three-fold replication was conducted throughout the experiment. After certain exposure time with latex, the cytotoxicity of latex was evaluated by MTT assay. The cytotoxicity data was obtained from the OD of cells which were exposed to diluted latex subtracted by the OD of diluted latex.

## RESULT

Optimization of FBS concentration in medium showed that with 10% FBS there was more precipitate formed than with 5% FBS, while 0% FBS showed lesser cell number compared to those of 10%. OD at 540 nm of cells in medium supplemented with 10% FBS and treated with latex 15 to 1000  $\mu\text{g/ml}$  ranged from 0.648 to 0.351. While for the cells in medium treated with 5% FCS, the OD ranged from 0.730 to 0.407. Moreover, samples with 5% FBS was easier to manipulate, because there was less precipitate formed. Therefore, medium supplemented with 5% FBS was used for experiment. Optimization for concentration of latex from 36 to 150,000  $\mu\text{g/ml}$  showed that there were significant difference between 1100  $\mu\text{g/ml}$  and 2300  $\mu\text{g/ml}$ . The OD was nearly 0 at 2300  $\mu\text{g/ml}$  latex, while at 1100 the OD was around 0.500. Then we chose 5000  $\mu\text{g/ml}$  latex in medium as the highest concentrations for experiment. MTT assay of Fib L929 cells after exposure to different concentration of latex showed that the OD was lowered at 312  $\mu\text{g/ml}$  latex and became half of that of control (0  $\mu\text{g/ml}$  latex) at 625  $\mu\text{g/ml}$ . No living cells were observed at latex of 2500  $\mu\text{g/ml}$  (figure 2). The various time of exposure which was 1, 2, and 3 days at the same concentration showed no difference at OD values. Cytotoxicity in human gingival fibroblast showed no living cells at 1200  $\mu\text{g/ml}$  latex, and OD became half of that of control at 150  $\mu\text{g/ml}$  (figure 3).

## DISCUSSION

The aim of this study was to explore the mechanism of how dental pulpal pain disappeared with no recurrence if *J. curcas* latex is put into the cavity formed in dental caries to have a direct contact with

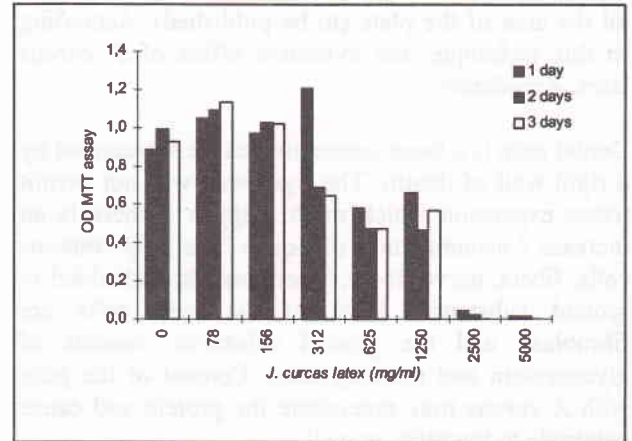


Figure 2. Graph showed the OD (540 – 690 nm) of cytotoxicity test of *J. curcas* latex by MTT assay using Fib L929. At  $\mu\text{g/ml}$  latex the cells were half of that of control (0  $\mu\text{g/ml}$ ) and no living cells at 2500  $\mu\text{g/ml}$

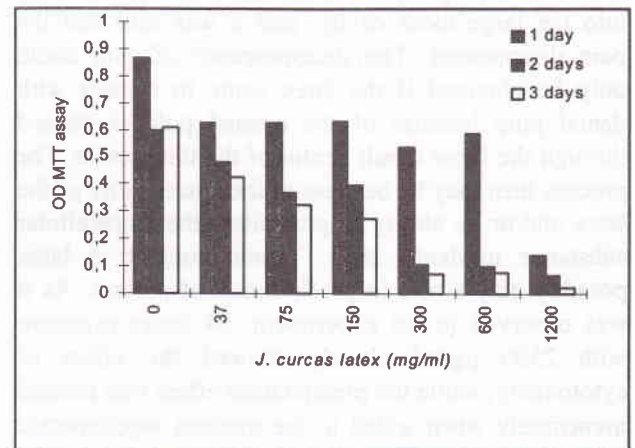


Figure 3. Graph of OD (540 – 690 nm) of cytotoxicity test of *J. curcas* latex by MTT assay using human gingival cells. At day 2, the amount of cell was half of that of control at 150  $\mu\text{g/ml}$  latex, while was no living cells was observed at 1200  $\mu\text{g/ml}$  latex

the pulp. One possibility is that the latex is cytotoxic, hence, the pulp in contact will be necrotized, and as a consequence there will be no more pain.

The experiment showed that the latex was cytotoxic to fibroblast cell line and primary cell culture. The cytotoxic effect was obtained in low concentration, 1250 - 2500  $\mu\text{g/ml}$ , compared to the concentration of fresh latex which is 150,000  $\mu\text{g/ml}$ . Besides, it is found that the latex has the ability to precipitate the protein component of the medium. However, other cytotoxic experiments using agar overlay technique showed that the cytotoxic effect was limited to a certain zone surrounding the latex, and does not cover



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