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In Vitro Effect of Tetraprenyltoluquinone on Normal Human Leukocyte Cell

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ABSTRACT

Tetraprenyltoluquinone is a compound that was successfully isolated from the hexane fraction of stem bark *Garcinia cowa,* Roxb. In previous studies, tetraprenyltoluquinone is known to have anticancer and anti-inflammatory activity. The aim of the study is to investigate the impact of tetraprenyltoluquinone on normal human leukocyte cell cultures. Tetraprenyltoluquinone with a concentration between 3.125-1000 μg/mL was tested for cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and measured at λ 570 nm using a microplate reader. The results showed that tetraprenyltoluquinone was not toxic to human leukocyte cell cultures and had an IC_{50} of 2588.938 μg/mL.

Keywords: *Garcinia cowa, Roxb, leukocyte, MTT assay, tetraprenyltoluquinone.*

1. INTRODUCTION

Garcinia cowa, Roxb is a tree species that belongs to the *Clusianaceae* family and has been widely used as a traditional medicine. Locally it is known Asam Kandis among people of Indonesia. Previously *G. cowa* reported with good anti-bacterial activities, antiplatelet aggregation capacity, anticancer activity against human colorectal adenocarcinoma cells [1–4]. Tetraprenyltoluquinone is one of the active substances that has been isolated from the bark of this plant [5]. This compound showed several pharmacological activities such as cytotoxic, anticancer, and anti-inflammatory. Tetraprenyltoluquinone had selective cytotoxic activity against lung cancer cell culture (H-460) with IC_{50} value of 16.3±3.0 μM. This compound also had a strong inhibitory activity against NO with inhibitory value of 80.98% [6–8].

Leukocytes are innate immunity in the body. Leukocytes act as body's defense system against certain diseases and protect body from infection [9]. Leukocytes have been used to assess the toxicology of a plant extract or natural compound. Evaluation of genotoxic, cytotoxic and antioxidant activity of a natural compound against leukocytes yields well analyzed results [10].

Although tetraprenyltoluquinone have been reported as potential substances to be developed as drugs, there is a need for a more definitive clarification of the toxicity of tetraprenyltoluquinone particularly its cytotoxicity as well as safe concentrations and doses used. In this context, there is a need for research evaluating the toxicology of tetraprenyltoluquinone. Thus, this study aimed to predict the toxicity of tetraprenyltoluquinone, as well as its cytotoxicity in human leukocyte cultures.

2. METHODS

2.1. Cell Lines

Human blood sample was obtained from a healthy volunteer through vein. Blood sample used should be fresh, no more than 24 hours after collected from volunteer. 3 mL of blood was drawn, then rotated at 3000 rpm for 5 minutes. The plasma portion was removed and the remaining part of the cell was diluted with ammonium chloride buffer lysis solution (1:10), then homogenized for 10 minutes. After that, the buffer lysis suspension was rotated at 3000 rpm for 5 minutes and the liquid was removed. Lysis of the blood sample is repeated if there are still visible red blood cells. White blood cell pellets were resuspended in PBS and the cell pellets were washed to remove the lysis reagent. Then the sample was rotated at 3000 rpm for 5 minutes and the liquid was removed. Isolated leukocytes were resuspended in RPMI 1640 medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Leukocytes cell incubated for 24 hours at 37°C. The isolated cells were counted using hemocytometer by taking 10 μL of leukocyte cell suspension, put in a microtube, added with 10 μL of trypan blue, and then pipetting to make it homogeneous. After

that, take 10 µL of the mixture, placed in counting chamber, then counted under an inverted microscope [11].

2.2. Cytotoxic Assay

Leukocyte culture is harvested, and cells are counted. Total of cells required was 10^6 cells/mL in RPMI 1640 medium. Cells were distributed in 96-well plates with 10^4 cells per well tetraprenyltoluquinone was added at concentration of 1000; 600; 400; 100; 50; 25; 12.5; 6.25 and 3.125 µg/mL in each well (total volume 100 µL/well). Furthermore, plates were incubated with the compound at 37°C for 24 hours. After incubation, 20 µL of MTT solution (5 mg/mL) was added to each well. Then, cells were kept at 37°C for 6 hours. After that, the purple formazan salt formed was dissolved in 50 µL of DMSO. Negative control was untreated cell culture and positive control was leukocyte growth medium.[12] Absorbance was measured with microplate reader at wavelength of 570 nm.

2.3 Data Analysis

Data were calculated as mean ± SD from triplicate measurement. The IC_{50} value was determined by plotting the curve between the percent viability and concentration to obtain the regression equation. From the regression equation, it can be determined the amount of concentration that has an inhibitory ability of 50%.

3. RESULTS AND DISCUSSION

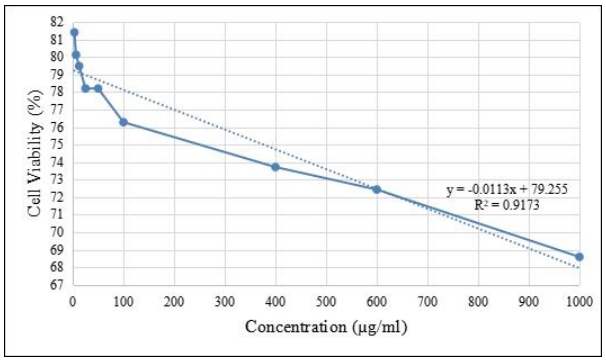
MTT assay used in measuring cell growth in response to mitogens, antigenic stimulation, growth factors, cytotoxicity studies and cell growth curve derivation. MTT tetrazolium salt was converted to purple formazan product by live cell proliferation [12]. The discoloration of yellow substrate becomes purple due to the active metabolism of living cells. When cells die, cells are unable to convert MTT to formazan, so the color change serves as an indicator of living cells [13].

The results of calculating the percentage of leukocyte cell viability (Table 1 and Figure 1) showed a decrease in percentage of cell viability along with the increasing concentration of tetraprenyltoluquinone. This indicates that this compound has an effect on the proliferation of living cells. The percentage of cell viability was verified based on absorption value obtained. It showed the number of leukocyte cells that still alive after induced with tetraprenyltoluquinone. Based on the results obtained, it appears that intensifying the level of tetraprenyltoluquinone does not increase cell death with an IC_{50} value of 2588,938 µg/mL. The greater IC_{50} value, indicates that the compound is less toxic [14].

Table 1. the effect of several concentrations of tetraprenyltoluquinone on leukocytes cell viability (%)

Concentration	Cell viability (%) ± SD
0	100.000 ± 7.772
3.125	81.410 ± 2.938
6.25	80.128 ± 1.923
12.5	79.487 ± 5.875
25	78.205 ± 5.088
50	78.205 ± 5.088
100	76.282 ± 5.769
400	73.718 ± 11.268
600	72.436 ± 8.813
1000	68.590 ± 11.698

Figure 1. Cytotoxic activity of tetraprenyltoluquinone toward leukocyte cell lines



A study stated that a pure compound is having a very active cytotoxic activity if it has an IC_{50} value <5 µg/mL. IC_{50} value of >50 µg/mL indicates that a pure compound has a weak cytotoxic activity, and if IC_{50} value >100 µg/mL indicates that the compound does not have cytotoxic activity against human leukocyte culture cells.

4. CONCLUSION

Based on this study it can be concluded that tetraprenyltoluquinone is not cytotoxic to human leukocyte cell cultures with an IC_{50} value of 2588.938 µg/mL.

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REFERENCES

[1]. Chouni A, Pal A, Gopal PK, Paul S. GC-MS analysis and screening of anti-proliferative potential of methanolic extract of Garcinia cowa on different cancer cell lines. Pharmacogn J. 2021;13(2). https://doi.org/10.5530/pj.2021.13.45

[2]. Siridechakorn I, Phakhodee W, Ritthiwigrom T, Promgool T. Fitoterapia Antibacterial dihydrobenzopyran and xanthone derivatives from Garcinia cowa stem barks. Fitoterapia. 2012;83(8):1430–4. https://doi.org/10.1016/j.fitote.2012.08.006

[3]. Sharma A, Joseph GS, Singh RP. Antioxidant and antiplatlet aggregation properties of bark extracts of Garcinia pedunculata and Garcinia cowa. J Food Sci Technol. 2014;51(8). https://doi.org/10.1007/s13197-014-1381-z

[4]. Chowchaikong N, Nilwarangkoon S, Laphookhieo S. p38 inhibitor inhibits the apoptosis of cowanin-treated human colorectal adenocarcinoma cells. 2018;2031–40. https://doi.org/10.3892/ijo.2018.4353

[5]. Wahyuni FS, Byrne LT, Dachriyanus, Dianita R, Jubahar J, Lajis NH, et al. A new ring-reduced tetraprenyltoluquinone and a prenylated xanthone from Garcinia cowa. Aust J Chem. 2004; https://doi.org/10.1071/CH03175

[6]. Wahyuni FS, Stanslas J, Lajis NH, Dachriyanus. Cytotoxicity studies of tetraprelyltoluquinone, a prenilated hydroquinone from Garcina cowa Roxb on H-460, MCF-7 and DU-145. Int J Pharm Pharm Sci. 2015;7(3):60–3.

[7]. Wahyuni FS, Hui LS, Stanslas J, Lajis NHJ, Dachriyanus. In vivo study of tetraprenyltoluquinone, an anticancer compounds from garcinia cowa roxb. J Young Pharm. 2017; https://doi.org/10.5530/jyp.2017.9.58

[8]. Wahyuni FS, Ali DAI, Lajis NH, Dachriyanus. Anti-inflammatory activity of isolated compounds from the Stem Bark of Garcinia cowa Roxb. Pharmacogn J. 2017; https://doi.org/10.5530/pj.2017.1.10

[9]. Bozza PT, Melo RCN, Bandeira-Melo C. Leukocyte lipid bodies regulation and function: Contribution to allergy and host defense. Vol. 113, Pharmacology and Therapeutics. 2007. https://doi.org/10.1016/j.pharmthera.2006.06.006

[10]. Szeto YT, Lee KY, Kalle W, Pak SC. Protective effect of grape seed extracts on human lymphocytes: A preliminary study. Appl Physiol Nutr Metab. 2013;38(3). https://doi.org/10.1139/apnm-2012-0296

[11]. Wahyuni FS, Sudji IR, Amaliyah RA. Evaluasi Sitotoksik Alfa Mangostin Pada Kultur Sel Leukosit Manusia Secara In Vitro dan Uji Aktivitas Antioksidan. J Sains Farm Klin. 2019;5(3). https://doi.org/10.25077/jsfk.5.3.201-206.2018

[12]. Stockert JC, Blázquez-Castro A, Cañete M, Horobin RW, Villanueva Á. MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. Acta Histochem. 2012;114(8). https://doi.org/10.1016/j.acthis.2012.01.006

[13]. Kamiloglu S, Sari G, Ozdal T, Capanoglu E. Guidelines for cell viability assays. Food Front. 2020;1(3). https://doi.org/10.1002/fft2.44

[14]. Aykul S, Martinez-Hackert E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. Anal Biochem. 2016;508. https://doi.org/10.1016/j.ab.2016.06.025