บทความวิจัย (Research Article)

Cytotoxicity evaluation of leaf-extract from *Moringa oleifera, Cratoxylum formosum, and Mangifera indica*

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Abstract:

The study is set out to investigate the cytotoxic effect of three water leaf-extract from indigenous plants: *Moringa oleifera* (MO)*, Cratoxylum formosum* (CF)*, and Mangifera indica* (MI), using TK6 human lymphoblastoid cells (TK6 cell) as a model. The leaf-extract of CF showed the lowest toxicity among all extracts while the MI leaf-extracts showed the highest toxicity to TK6 cells. The half maximum inhibitory concentration (IC_{50}) of CF, MO, and MI were 381.48 ± 36.13, 198.21 ± 7.41, and 142 ± 22.87 µg/mL. The result suggested that water extracts of CF, MO, and MI have low cytotoxicity towards TK6 cells.

Keywords: Cytotoxicity test, WST-1 assay, *Moringa oleifera, Cratoxylum formosum, Mangifera indica,* TK6 cells

Introduction

Moringa oleifera (MO)*, Cratoxylum formosum* (CF)*,* and *Mangifera indica* (MI) are indigenous Thai vegetables, grown in the North and North-East of Thailand. Various parts of the plants such as fruit, flower, and leaf are used for health benefit. Generally the leaves of these plants are traditionally consumed in Thailand. It has been reported that the leaf of these plants show anti-oxidant property due to their chemical constituents, especially polyphenolics and flavonoids. [1-4] This may be beneficial to several chronic disorders such as cardiovascular disease, Alzheimer's disease and cancer. Therefore, these leaves are commonly used as a food intake, or nutritional supplement and traditional medicine for humans. [5] Although these plants are consumed for long periods of time by many countries worldwide due to their bioactivity attributes. There have been reports that the consumption can lead to adverse effects.

The cytotoxicity profile is not well documented. Thus, the objective of the study was to ascertain possible toxicity of water extracts from leaves of MO, CF, and MI at cellular levels using a metabolic activity-based WST-1 assay. The assay is rapid and can be performed entirely in a microliter plate and is now widely used for quantification of cell proliferation and cytotoxicity. [6-7]

In principle, the water-soluble tetrazolium salt (WSTs), WST-1 works similarly to the conventional methyl tetrazolium 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay by reacting with mitochondrial succinate-tetrazolium reductase forming the formazan dye. Different from MTT assay, the WST-1 method produces a water-soluble formazan rather than the water-insoluble product of the MTT assay. Therefore, the WST-1 reaction product can be quantified in 0.5 to 4 h without an additional solubilization step. [8]

Material and Method

Extraction preparation

Fresh leaves of MO, CF, MI were harvested and collected from Phayao Province, North of Thailand in December 2012. The leaves were cleaned with tap water and air-dried. Two hundred-fifty grams of fresh leaves were blended with 2 L distilled water and heated for 1 h at 80˚C in a water bath. After, filtration through Whatman no.1 filter paper using a suction apparatus, the extract was lyophilized. The dry extracted was weight and kept at -20˚C. The yield of freeze-dried powder from fresh leaves was about 3.14%, 8.21%, 6.32% weight by volume (W/V), for MO, CF, and MI extracts, respectively. [9]

Cell culture and maintenance

The stock TK6 human lynphoblastoid (TK6) cell line (CRL-8015) was purchased from American Type Culture Collection (ATCC) in Maryland, US. Cells were grown in suspension and maintained as exponential phase in Roswell park memorial institute (RPMI) 1640 medium supplemented with 10% heat-inactivated horse serum (HS) and incubated at 37˚C in an incubator containing 5% CO_2. Cells were sub-cultured every 48 h.

WST-1-based cytotoxicity assay

The microplate WST-1 assay was performed as follows, After cultured overnight, TK6 cells at a density of 1×10^4 cells/mL were exposed to extracts (individual MO, CF, MI) in the 6-well plate at various concentrations RPMI for 24 h under conditions mentioned above. After treatment, the test chemical-containing medium was removed by centrifugation at 1542.75 xg for 5 min. The treated cells were gently washed at least twice with hank balanced salt solution (HBSS). Then the cells were re-suspended with fresh RPMI and analyzed for cytotoxicity effects. 200 µL of treated cells were pipetted to a 96-well plate, and then 10 µL of reagent WST-1 was added to each well and incubated at 37% under 5% CO_2 in an incubator for 30 min. The absorbance was measured in a microplate reader at 430 nm and the percent cytotoxicity was calculated.

Statistical analysis

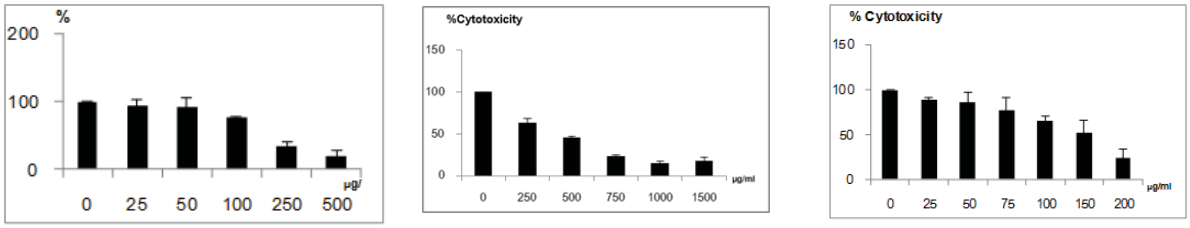
Each result is expressed as mean ± standard deviation (SD), and the data were analyzed by one-way ANOVA, using SPSS software program (SPSS 11, USA). The differences between samples were determined by Sheffe test. The results were considered to be significant at less than 0.05. The half maximum inhibitory concentration (IC_{50}) values were calculated from linear regression analysis.

Results

The extracts exhibited low activity against TK6 cells, achieving IC_{50} values of 198.21 ± 7.41 (MO), 381.48 ± 36.13 (CF), and 142.25 ± 22.87 (MI) µg/mL, respectively. Moreover, the number of dead cells increased correspondingly to the concentration of the extracts used in the treatments.

The MO revealed the proportion of TK6 cell survival of 95.52, 92.31, 75.85, 34.86, and 18.42%, respectively, at 25, 50, 100, 250, and 500 µg/mL (Figure 1a). The CF had lowest cytotoxicity of 62.94, 45.81, 23.47, 14.67, and 17.67%, respectively, at 250, 500, 750, 1000, and 1500 µg/mL, respectively (Figure 1b), compared to MI of 88.77, 86.50, 77.24, 64.65, 51.51, and 24.17% at 25, 50, 75, 100, 150, and 200 µg/mL, respectively (Figure 1c).

There were no significances among the corresponding concentrations tested using Sheffe test.



1a 1b 1c

Figure 1 Cytotoxicity effects of M. oleifera (1a), M. indica (1b) and C. formosum (1c)

Discussion

The MO, CF, and MI leaf-extract had low cytotoxic effects (IC50) against TK6 cells at the maximum concentration between 500 and 1,500 µg/mL, and were similar to the Nigerian study of MO between 100 and 1,000 µg/mL, and safe in mice. [10] In addition, the safe dose of MO leaf-extract is as high as 5,000 mg/kg. [11] While some traditional plants had moderate cytotoxicity between 100 and 1,000 µg/mL. [5]

The mechanism of *in vitro* of CF on human leukemia U937 cell is the induction of apoptosis (programmed cell death) via pro-apoptosis protein [12]. The present study provided preliminary data showing low cytotoxic activity, however the further study in animal is required.

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