The Thai Journal of Pharmaceutical Sciences

Volume 36
Issue 0 *supplement issue*

Article 17

1-1-2012

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KEYWORDS *Spinacia oleracea*, cytotoxicity, Hepatocarcinoma, MTT assay

INTRODUCTION

Spinacia oleracea L. is an edible flowering plant in the family of Amaranthaceae. It is native to central and southwestern Asia. Its commonly known the name of Spinach. It also has a high nutritional value and rich in antioxidants, especially in fresh, steamed, or quickly boiled. Spinach is most often used as a food including medicinal value. Spinach contains an impressive range of active substances which are indispensable to the body. In its structure we find mineral salts (sodium, potassium, calcium, phosphorus, magnesium, sulfur, iron, zinc, manganese, iodine, copper), vitamins (C, B1, B2, B6, PP, E, K, folic acid, vitamin A), chlorophyll, amino acids such as arginine and lysine, but also lipids and fibers. Iit is a good source of chlorophyll, beta-carotene and lutein. Alimentary fibers, vitamins and minerals contained make spinach a good depurative and detoxifier. The toxins originating in nourishments that are rich in fat and proteins of animal origin are thus eliminated quickly. At the same time, alimentary fibers prevent constipation and colon cancer. They interact with the absorption of fat and adjust the level of cholesterol in the body. Moreover, it has been used in Thai traditional medicine for relief to gastrointestinal disorder and hypoglycemic properties, urinary calculi, febrile conditions, lung and bowels inflammation. This study was performed to evaluate cytotoxicity of the powder of *S.oleracea* using MTT assay that was a part of scientific aspects.

MATERIALS AND METHODS

Sample preparation: The powder leaves of *S.oleracea* were weighed and dissolved in 100% dimethylsulfoxide making stock concentration of 100 mg/ml. The samples were then filtrated through a 0.2 um filter and were serial diluted in the culture medium of cells at a ratio of 1:2 giving 8 concentrations of 1000, 500, 250, 125, 62.50, 31. 25, 15.625 and 7.81 μg/ml.

Cell culture: The cells were HepG2 cell line (human liver hepatocarcinoma ATCC Cat. No.HB-8065). The cells were grown in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 0.1mM MEM non-essential amino acid, 1.0 mM Sodium pyruvate, 2mM L-glutamine and 100 unit/ml penicillin and streptomycin. The cells were incubated at 37°C in a fully humidified, 5% CO_2 : air atmosphere.

MTT cytotoxicity test(1-2) : MTT assay is a tetrazolium-dye based colorimetric microtitration assay. Metabolismcompetent cells are able to metabolize the tetrazolium (yellow) to formazan (blue); this color change is measured spectrophotometrically with a plate reader. It is assumed cells that are metabolically deficient will not survive, thus the MTT assay is also an indirect measurement of cell viability. The cells were seeded in a 96-well plate at a density of 5,000 cells/well, and incubated for 48 hours. The sample at various concentrations were added to the cells and incubated for 24 hours. The test samples were removed from the cell cultures and the cells were reincubated for a further 24 hours in fresh medium and then tested with MTT assay. Briefly, 50 μl of MTT in PBS at 5 mg/ml was added to the medium in each well and the cells were incubated for 4 hours. Medium and MTT were then aspirated from the wells, and formazan solubilized with 200 μL of DMSO and 25 μl of Sorensen's Glycine buffer, pH10.5. The optical density was read with a microplate reader (Molecular Devices) at a wavelength of 570 nm. The average of 4 wells was used to determine the mean of each point. The data were analyzed with the SoftMax Program (Molecular Devices) to determine the IC_{50} for each toxin sample. A dose-response curve was derived from 8 concentrations in the test range using 4 wells per concentration. Results of toxic compounds are expressed as the concentration of sample required to kill 50% (Inhibitory concentration 50; IC_{50}) of the cells compared to controls.

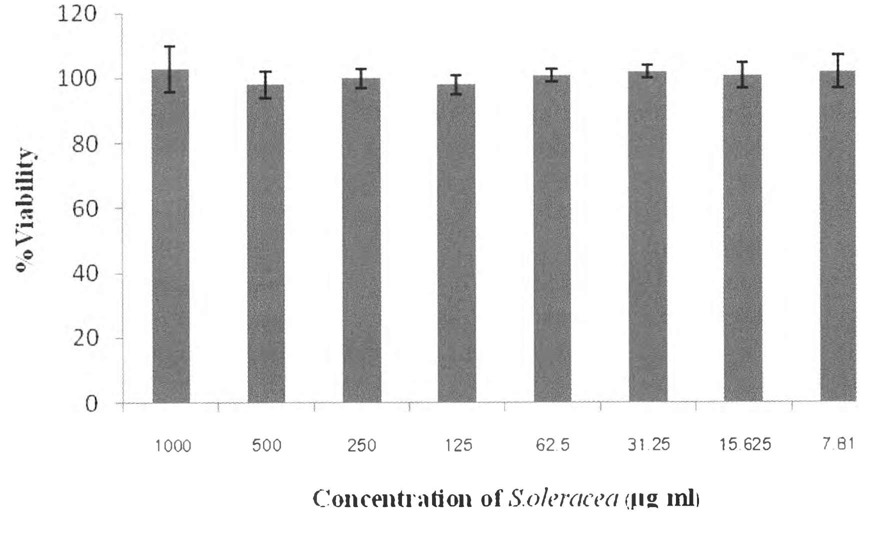
RESULTS AND DISCUSSION

The cytotoxicity test was shown in Table 1 and Fig. 1. The treatment of HepG2 cells for 24 hr with various concentrations of *S.oleracea* powder at 1000, 500, 250, 125, 62.50, 31. 25, 15.625 and 7.81 μg/ml. indicated that the IC_{50} value was more than 1000 μg/ml for treatment times.

Table 1: Viability of HepG2 following exposure to *S.oleracea* powder

Sample	Concentration (μg/ml)	: % Viability	IC50(μg/ml)
S.oleracea	1000	103±7	>1000
	500	98±4	
	250	100±3	
	125	98±3	
	62.50	101±2	
	31.25	102±2	
	15.625	101±4	
	7.81	102±5	

Figure 1: The Viability of HepG2 following exposure to *S.oleracea* powder



CONCLUSION

This assay was a modified version of conventional direct and indirect contact tests conformed to the published standard methods (BS-EN30993-5 and ISO10993-5). The MTT assay is a tetrazolium-dye based colorimetric microtitration assay. Metabolism competent cells are able to metabolize the tetrazolium (yellow) to formazan (blue); this color change is measured spectrophotometrically with a plate reader. It is assumed cells that are metabolically deficient will not survive, thus the MTT assay is also an indirect measurement of cell viability. The cytotoxicity results showed the % survival of HepG2 cell line, at each concentration compared to control and IC50 values, over the test concentrations of 1000-

7.81 ug/ml. The results showed that *S.oleracea* powder was not cytotoxic to HepG2 cell line over the tested concentrations.

ACKNOWLEDGEMENT

The author would like to gratitude thanks to Thailand Institute of Scientific and Technological Research for their financial support.

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