Evaluation of Silibinin on the Viability of MCF-7 

Human Breast Adenocarcinoma and HUVEC 

(Human Umbilical Vein Endothelial) cell lines

Maliheh Entezari¹, Mohammad Javad Mokhtari² and Mehrdad Hashemi³

1. Department of Genetics, Tehran Medical Branch 
   Islamic Azad University Tehran, Iran 
   (*corresponding author, e-mail: Entezarimali@yahoo.com)

2. Department of Biology, Zarghan Branch 
   Islamic Azad University, Zarghan, Iran

3. Department of Genetics, Tehran Medical Branch 
   Islamic Azad University, Tehran, Iran

Abstract. Breast cancer comprises 10.4% of all cancer incidences among women, making it the most common type of non-skin cancer in women and the fifth most common cause of cancer death. Silibinin, also known as silybin, is the major active constituent of silymarin. Silibinin has also demonstrated anticancer effects against human prostate adenocarcinoma cells, estrogen-dependent and – independent human breast carcinoma cells, human ectocervical carcinoma cells, human colon cancer cells, and both small and nonsmall human lung carcinoma cells. In the present study, MCF-7 and HUVEC cells were incubated with different concentrations of silibinin (100μg/ml, 150μg/ml and 200μg/ml) at 24h. Then cell cytotoxicity was assessed by MTT assay and IC50 was determined by Pharm software. The results showed that silibinin has dose-dependent inhibitory effect on the viability of MCF-7 cells and with the least inhibitory effect on the viability of normal cells (HUVEC).
Keywords: Silibenin; Breast Cancer; MCF-7; HUVEC; Normal Cell

INTRODUCTION

Nowadays cancer is one of the mortality factors in the world which takes place in result of different causes such as mutagenesis and carcinogen chemicals in the environment. Environmental agents which serve as mutagens are cancer factors. According to the statistics almost more than 75% of cancers have an environmental origin (1, 2). Genetic damages and changes in DNA sequences and genes mutations and other changes in chromosomal structure play an important role in cancer (3). Most of mutagenic and carcinogen agents display their destructive effects through free radicals including reactive oxygen’s species (ROS). So that antioxidants are able to reduce ROS. ROS have a role in etiology of diseases such as cancer, cardio cellular, nerves problems and senescence. So daily consumption of antioxidants enhances immunity of the body against free radicals production and serves as anticancer agent (4, 6). Some of the fruits and vegetables are considered as the main anticancer foods, because of their abundant antioxidants such as phenols, vitamin C, vitamin E, beta-carotene and lycopene (7). The polyphenolic phytochemical silibenin, a flavonolignan, is a major constituent of the seeds of milk thistle (Silybum Marianum). Silibenin and silymarin, a standardized milk thistle extract of which silibenin is a major component, are widely consumed as dietary supplements, especially in the USA. Silibenin is the most interesting one among these antioxidants (8, 9). This research has been tried to consider anticancer effects of Silibenin on cancerous and normal cells.

Materials and methods

Cell culture and silibenin treatment

MCF-7 and HUVEC cell lines were obtained from the National Cell Bank of Iran (NCBI). The cells were cultured in DMEM containing 10% FBS, 2 mM glutamine, antibiotics (penicillin G, 60 mg/L; streptomycin, 100 mg/L; amphotericin B, 50 μL/L) under a humid atmosphere (37°C, 5% CO2, 95% air). For silibenin treatment, appropriate amounts of stock solution [1mg/ml in dimethyl sulfoxide (Merck, Darmstadt, Germany)] of silibenin (Sigma, USA) were added into culture medium to achieve the indicated concentrations and then incubated with cells for 24h, whereas dimethyl sulfoxide solution without silibenin was used as blank reagent.
Determination of cell viability

The effect of silibinin on MCF-7 cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, USA) assay. Briefly, 10^4 cells/well were treated with various concentrations of silibinin (0, 100, 150 and 200 μg/ml). After 24 hour incubation the cells were washed twice with phosphate buffered saline (PBS) and MTT (0.5 mg/mL PBS) was added to each well and incubated at 37°C for 3h. The formazan crystals that formed were dissolved by adding dimethyl sulfoxide (100 μL/well), and the absorbance was read at 570 nm using a microplate scanning spectrophotometer (ELISA reader, Organon Teknika, Netherlands). Toxicity level was calculated by the following formula:

\[ \text{Cytotoxicity} \% = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100 \]

Viability % = 100 - Cytotoxicity %

To diminish test error level, MTT strain was added to some wells without cells and along with other wells, absorbance level was read and ultimately subtracted from whole the absorbance.

IC50 determination

The 50% inhibition concentration (IC50) values of silibinin on MCF-7 and HUVEC cells at 24h were determined. IC50 was determined by probit analysis using the Pharm PCS (Pharmacologic Calculation System) statistical package (Springer-Verlag, USA)

Statistical analysis

Statistical significances of difference throughout this study were calculated using a Student s t-test and by one-way variance analysis.

Results

Silibinin cytotoxicity on MCF-7 and HUVEC cells

Different concentrations of silibinin (0, 100, 150 and 200 μg/ml) at 24h have cytotoxicity effects on MCF-7 and HUVEC cell lines. Compared to the controls, different doses of silibinin (100μg/ml) – 10.625% and 96.843%, (150μg/ml) – 7.571% and 91.104%, (200μg/ml) 4.334% and 81.434 - decreased MCF-7 and HUVEC cells number, respectively (P<0.05) (Fig. 1)
The IC50 silibinin after 24h for MCF-7 and HUVEC cell lines was calculated 15.771µg/ml and 387.4156µg/ml, respectively (P<0.05). There was a significant difference between Silibinin effect on growth depression of MCF-7 and HUVEC cells (P<0.01).

Discussion

Since usual methods on cancer treatment (surgery, chemical treatment, radiotherapy) have an effect on natural dividing cells, in addition to tumor cell, and kill or arrest their cell division(10). Interest in naturally occurring products is increasing for the prevention of carcinogenesis. Based on this idea, certain foods such as vegetables, fruits, and grains, as well as other phytotherapeutic agents offer high anticancer efficacy and low toxicity to normal tissue. One such dietary agent is silibinin, which has a wide range of pharmacological effects, such as inhibition of DNA synthesis, cell proliferation, cell cycle progression, and apoptosis in various cancer cell lines, including breast cancer. Moreover, the administration of this compound to various animals has been shown to be nontoxic in many studies (11). In 1999, Bhatia et al. reported that treating of prostate, breast, and cervical carcinoma cells with silibinin results in a highly significant inhibition of cell growth and DNA synthesis in a time-dependent manner with a large loss of cell viability only in the case of cervical carcinoma cells. In 2011, Noh et al. founded that silibinin induced apoptotic cell death in MCF-7 cells. Angioprevention and antiangiogenic therapy are considered as efficient strategies for controlling the growth and metastasis of solid tumors as well as for other diseases involving pathological angiogenesis. Singh (2005) showed that silibinin exhibits pleiotropic antiangiogenic effects in human endothelial cells. They also observed that silibinin (a) inhibits capillary tube formation on matrigel;
(b) disrupts preformed capillary network; (c) inhibits matrigel invasion and migration; and (d) inhibits MMP-2 secretion by HUVEC. In summary, pleiotropic antiangiogenic effects of silibinin involves growth inhibition, cell cycle arrest, apoptosis induction, inhibition of capillary tube organization, reduced invasion and migration of human endothelial cells. In this study, we observed that silibinin has dose-dependent inhibitory effect on the viability of MCF-7 and HUVEC cell lines.

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