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Anti-Cancer Activity of Methanol Extracts of Cichorium Intybus on Human Breast Cancer SKBR3 Cell Line

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Abstract

Background: Breast cancer is the most prevalent cancer and the second cause of death among women around the world. In many cancers, including breast cancer, Fatty acid synthase (FASN) gene expression is increased significantly. In breast cancer cell lines, expression of FASN is higher in HER2 positive cell line like SKBR3 than the others. FASN is the key enzyme for fatty acid synthesis de novo pathway and it is producing palmitate which is necessary for cell membrane formation. Cichorium intybus is a medicinal plant that effectively leads to inhibition of fatty acid synthase and thus reduces the percentage of survival of cancer cell lines.
Objectives: The aim of this study was to evaluate the effect of methanol extract of Chicorium intybus root on percentage of survival in SKBR3 cell line.
Methods: Human breast cancer SKBR3 cell line was cultured in DMEM medium. Methanol extract of Cichorium intybus root was extracted and different dilutions (200, 300, 400, 500 and 600μg/mL) were added to cell culture. Cell viability was quantitated by MTT assay after 24, 48 and 72 hours.
Results: Cichorium intybus could decrease cell viability. The effects of extract on cell viability were observed after 24, 48 and 72 hours on SKBR3 cell line and IC50 was 800, 400 and 300 after 24, 48 and 72 hours of treatment, respectively.
Conclusions: Our study shows that methanol extract of Cichorium intybus has cytotoxic effects on tumor cells. This is a pilot work for further evaluation in the future.

Keywords: Cichorium Intybus, SKBR3 Cell Line, Fatty Acid Synthase, MTT Assay, Cell Viability

1. Background

Lipids are essential part of cell membrane and they are also necessary for cell proliferation. First synthesized lipid is palmitate (16c) and the other types of lipids which are needed for cell membrane comes from it. Fatty acid synthase (FASN) is the key enzyme for fatty acid synthesis de novo pathway and it is producing palmitate by compounding acetyl-coA, malonyl-coA and consuming NADPH (Figure 1) (1).

FASN has a low expression in normal tissue cells but when normal cells are removed from normal conditions and goes toward cancerous, lots of metabolism changes occur in them and let these cells to have faster growth and higher proliferation rate. One of the most important changes in cancer cell lines is the high expression and high activity of fatty acid synthase enzyme. So FASN become overexpressed in many cancerous tissue cells like: breast, prostate and colon compared to surrounding normal tissue. This change of the cancerous cell lines also known as a sign for prognosis of tumor aggression (2).

Fatty acid synthase gene expression in various cancer cell lines is different. Among human breast cancer cell lines, SKBR3 has the highest expression of the enzyme. SKBR3 is ER^- PR^- and $HER2^+$ with metastatic ability, like: MDA-MB-231 and MDA-MB-468. But it is in contrast to MCF-7, which is ER^+, PR^+ and $HER2^-$ with no metastatic ability.

De novo fatty acid synthesis pathway in cancer cell lines let them to synthesize more fatty acids and so, have faster growth and higher proliferation. Therefore, FASN inhibition can be an effective way for cancer restriction or even treatment. Although the underlying mechanism is still under studying, several theories have been proposed. For example: fatty acid synthase inhibitors such as methanol extract of chicory can reduce the amount of fatty acids which are needed for cell membrane formation and this leads to reduce the cell growth and proliferation rate. An alternative view suggested that malonyl-coA accumulation in the cell prepared the condition for cell apoptosis (3, 4).

Cichorium intybus is a small herb from Asteraceae family which is also known as chicory. This plant has 1 meter height with relatively flat leaves and geographically dispersed all over the world but mostly in European and Asian countries (mostly in Iran) grows (5). Chicory known as a

Figure 1. The Synthesis of Palmitate by Fatty Acid Synthase

I. $7\ \text{Acetyl-CoA} + 7\ Co_2 + 7\ \text{ATP} \longrightarrow 7\ \text{Malonyl-CoA} + 7\ \text{ADP} + 7P_i$

II. $1\ \text{Acetyl-CoA} + 7\ \text{Malonyl-CoA} + 14\ \text{NADPH} + 14H^+ \longrightarrow \text{Palmitate} + 8\ \text{CoA} + 7\text{ADP} + 14\text{NADP}^+ + 6\ H_2O$

III. $8\ \text{Acetyl-CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 14\ H \longrightarrow \text{Palmitate} + 8\ \text{CoA} + 7\text{ADP} + 7\ P_i + 14\ \text{NADP}^+ + 6\ H_2O$

I: Malonyl-CoA synthesis by acetyl-coA carboxylase (ACC). II: Palmitate synthesis from Acetyl-CoA and Malonyl-CoA by FASN. III: Overall equation, combination of, I and II.

medical herb in Eurasia and different parts of it (root, stem and leaf) are used as an anti-diabetic, anti-viral disease, anti-malaria and also anti-cancer compound. Also, whole plant of chicory traditionally used for stomachic discomfort, hypotension and as a laxative (6).

One of the studies that have been done recently on chicory is prepared methanol and aqueous extract from different part of this plant (root, stem and leaf) then review and analysis extract by techniques like HPLC. These studies show that, extract of chicory included compound such as: Lactucin, β-Sitosterol, Quinic acid, Succinic acid and polyphenols like flavonoids (7). These compounds can be found in all parts of the plant, but most of them are accumulated in roots and a higher percentage of them are achieved by methanol extract compared to aqueous extract (8).

Flavonoids have an antitumor effect because of their fatty acid synthase inhibition ability (9).

2. Objectives

The aim of the present study is to evaluate cytotoxic effect of methanol extract of Cichorium intybus on human breast cancer SKBR3 cell line.

3. Methods

3.1. Plant Material

Chicory roots were collected from around Kashan, Iran and were identified by traditional medicine research center, Shahid Beheshti University of Medical Sciences. Roots were washed with distilled water and placed in an environment away from direct sunlight to dry and then dried roots were ground into fine powders and stored at room temperature for later use.

3.2. Methanol Extraction

Chicory root extraction was done by maceration method. 400 gram of powdered chicory root shed in a suitable container and then 4000 mL methanol 80% was added to it and placed on a shaker for 24 hours. In the following, yield extract was filtered by filter paper and condensed by rotary evaporator. Extraction of chicory root carried out in three stages, 24 hours. To prepare the stock of extract: 350 mg was dissolved in 1 mL DMSO. Different concentrations 200, 300, 400, 500, 600 and 700 μg/mL were prepared from stock for later use.

3.3. Cell Line And Cell Culture

SKBR3 cells were purchased from Pasteur Institute and then cultured in DMEM medium, containing: 10% fetal bovine serum (FBS) and 1% penicillin/ streptomycin and were incubated at 37°C in a humidified incubator with 5% CO_2.

3.4. Mtt Assay

To determine the toxicity of this extract, MTT assay was done according to ATCC protocol. SKBR3 cells were trypsinized and seeded in 96-well plates at the density of 5×10^3 cells/well. At 40% - 50% confluency (24 hours after seeding), cells were treated for 24, 48 and 72 hours with different concentrations of methanol extract of chicory (200, 300, 400, 500, 600μg /mL) against control (1% DMSO without any treatment). Eventually, determination of cell viability was done by MTT assay and using Elisa reader by observing absorbance at 570 and 630 nm wavelengths.

3.5. Statistical Analysis

MTT assay was performed in triplicate, biologically and technically. Each concentration was repeated three times per plate and also 24, 48 and 72 hours was performed as a triplicate.

Results were compiled to excel program. One-way ANOVA and Post Hoc tests were applied for data analysis. $P < 0.05$ data were considered statistically significant.

4. Results

Methanol extracts of chicory decrease the viability of human breast cancer SKBR3 cell line.

This extract decreases the cell viability of SKBR3 cells in a time, concentration-dependent manner. As the dose of chicory extract and duration of treatment with this extract increased the percentage of cell viability decreased. Reduction of SKBR3 cell viability after 24 hours incubation with methanol extract of chicory is meaningless compared to the control. After 48 hours, chicory extract with 400, 500 and 600 μg/mL concentration, cause two-fold reduction in SKBR3 cells viability and as expected, after 72 hours incubation, these concentrations of chicory extract cause the highest reduction (more than two-fold) in SKBR3 cell line viability compared to the control group (Figure 2).

The results show that IC50 on SKBR3 cells was 800, 400 and 300 after 24, 48 and 72 hours of treatment, respectively.

5. Discussion

Breast cancer is the most prevalent cancer and the second cause of death among women around the world (10). Breast cancer is a multifunctional disease which is also affected by environmental factors (11). However, despite extensive research in the field of breast cancer, the factors contributing to the progression of the disease and its molecular mechanisms are not fully understood (12). In the past, the most important treatment of breast cancer was surgery, chemotherapy, radiation or a combination of these methods. But in recent years, understanding of the molecular mechanisms involved in cancer pathways, leading to the creation of a new therapeutic approach, in which the most basic molecules that regulate metabolic pathways will be targeted (13). Various chemical compounds with the aim of influencing on these molecules (such as fatty acid synthase) and decrease cancer cells viability are used but yet, herbal compounds are more noteworthy, because of their fewer side effects. Therefore, this study is planning for evaluate the effect of methanol extract of Cichorium intybus root on SKBR3 cell viability, which is shown in Figure 2.

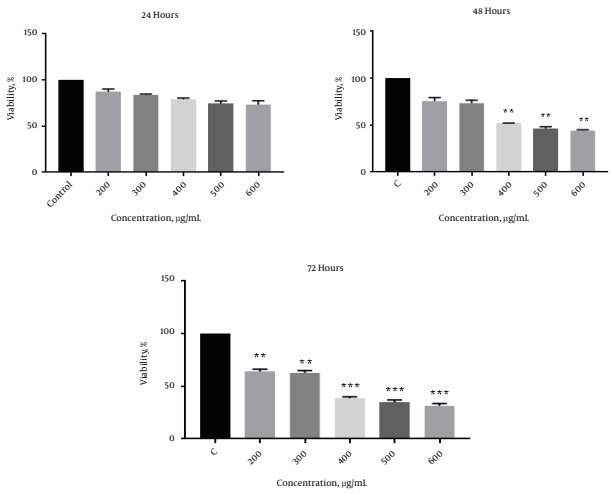
Given to the role of fatty acid synthase enzyme in supply necessary lipids for cell membrane formation, is anticipated that inhibition of this enzyme by methanol extract of Cichorium intybus root lead to decrease the viability of SKBR3 cell line (5, 14). Our results are in agreement with those reported below. According to Kummalue and colleagues, Erycible elliptilimba extract significantly reduced survival in SKBR3 human breast cancer cell line (15).

In a study, Salem and colleagues treated Jurkat cell line (human lymphoblastic leukemia) with extract of Cichorium intybus aerial parts. After 24 hours incubation, evaluation of cells by Trypan blue and MTS assay show decrease in Jurkat cell line viability in treated group compared to the control (G = 105). Our study results show that, percentage of survival of SKBR3 cell line decrease after 48 and 72 hours incubation with methanol extract of Cichorium intybus root.

5.1. Conclusion

In total, according to the results of this study and noted, the use of methanol extract of chicory root can reduce the percentage of survival in human breast cancer SKBR3 cell line. However, understanding of the basic mechanism of this change according to inhibition of fatty acid synthase, need more investigate in this area.

Figure 2. SKBR3 Cell Line Viability in Different Concentration of Methanol Extract of Cichorium Intybus (24, 48 and 72 Hours) Against Control (1%DMSO Without Any Treatment)



**P Value < 0.01, *** P Value < 0.001.

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