

Effects of *Rhamnus frangula* Extract on MCF7 Cells in Cell Culture

Shokoohian B, Ahmadi R, Molseghi M, and Mahdavi E

Abstract- Studies show that some plant extracts have anticancer effects on breast cancer cells. MCF-7 is a cell line that was first isolated in 1970 from the breast tissue. The aim of this study was to determine the effects of *Rhamnus frangula* extract on MCF7 cell viability in cell culture. In this laboratory experimental study, we used MTT assay to determine cell viability following administration of different doses (0.01mg/ml, 0.1mg/ml, 1mg/ml and 10mg/ml) of *Rhamnus frangula* in cell culture. The data were statically analyzed using ANOVA. The results showed that the administration of different doses of *Rhamnus frangula* resulted in decreased viability of MCF7 cells ($P < 0.05$). The higher the dose of extract was administered, the lower viability in MCF7 cells was observed. Our findings indicate that *Rhamnus frangula* extract has cytotoxic effects on breast cancer cells in cell culture.

Index Terms- Rhamnus frangula, MCF7, Viability

I. INTRODUCTION

Rhamnus frangula (figure 1), the alder buckthorn, is a tall deciduous shrub in the family Rhamnaceae. Alder Buckthorn was first reported by Linnaeus in 1753 as *Rhamnus frangula* [1]. Alder buckthorn is native to Europe, northern Africa, and central Asia. The first known North American collection occurred in 1898 in London, Ontario [2]. *Rhamnus frangula* grows to 3-6 m, in the best conditions to 7 m in height. It is commonly multi-stemmed, but rarely a small tree with a stem diameter of 20 cm. The flowers are small, 3-5 mm diameter, star-shaped with five greenish-white acute triangular petals. The fruit is a small black berry, 6-10 mm diameter, ripening from green through red in late summer to dark purple or black in early autumn [3]. *Rhamnus frangula* inhabits a wide range of soil and soil moisture conditions. Although this plant has usually decorative applications, one study reported *Rhamnus frangula* shows antifungal activity [4], [5].



Fig 1 *Rhamnus frangula*

Bahareh Shokoohian (MSc) is with the Department of Biotechnology Faculty of Basic Sciences, Iranian Blood Transfusion Organization (IBTO), Tehran, Iran. (E-mail: Bshokoohian@yahoo.com).

Rahim Ahmadi (PhD) is with the Department of Physiology, Faculty of Basic Sciences, Islamic Azad University, Hamedan Branch, Hamedan, Iran. (E-mail: Rahahmadi2012@yahoo.com).

Majid Molseghi (MSc) is with the Department of Physiology, Faculty of Basic Sciences, Islamic Azad University, Hamedan Branch, Hamedan, Iran. (E-mail: m.molseghi@yahoo.com).

Edris Mahdavi (PhD student) is with the Department of Gardening, Faculty of Agricultural Sciences, Guilan University, Guilan (E-mail: Edris_mahdavi@yahoo.com).

Breast cancer (BC), specifically mammary carcinoma is potentially life-threatening malignancy, and the most common cause of death from cancer in women worldwide, with a lifetime risk of one in nine, and its prevalence is increasing. It represents around 30% of all cancer in females and approximately 40,000 deaths in the United States per year. Important advances have been made in detection and treatment, but it still remains a significant scientific and medical challenge [6], [7].

Side effects of allopathic drugs have led to increased emphasis on the use of medicinal plants as a source of anticancer medicines particularly against breast cancer cell lines all over the world. Recent studies show that there are plant extracts that have antiproliferative effects on breast cancer cell lines [8]-[10]. One research indicates plants from the family *Rhamnaceae* has cytotoxic effect on Hela and MDA-MB-468 tumor cells [11], and another study reported tumor-inhibitory activity of *Rhamnus frangula* extract against the P-388 lymphocytic leukemia in mice [12].

Despite considerable reports on inhibitory effects of plants in *Rhamnus* genus on cancer cells, there is not considerable report on the effects of *Rhamnus frangula* growing in East-Iran on cancer cells, especially MCF7 cells. The main aim of this study was to determine the effects of *Rhamnus frangula* extract on MCF7 (breast cancer) cell line viability in cell culture.

II. MATERIAL AND METHODS

A. Extract preparation

Rhamnus frangula extract was prepared and different concentrations of extract were used in our study.

B. Protocol of Study

We used MTT assay in this work to determine the effects of *Rhamnus frangula* extract on MCF7 cells viability in cell culture. Briefly, the procedure was carried out in the following steps:

DAY ONE: 100 μ l of cells was added into each well (96 well plates) and incubate at 37°C with 5% CO₂ overnight.

DAY TWO: The media was removed and extract was added and incubated at 37°C with 5% CO₂ overnight. For control, 10% FBS was added to media.

DAY THREE: extract was removed from media. 20 μ l of 5 mg/ml MTT was added to each well and incubated for 4 hours at 37°C. 150 μ l isopropanol was added and covered with tinfoil and agitate cells on orbital shaker for 15 min. Absorbance was read at 570 nm with a reference filter of 630 nm and recorded.

C. Statistical Analysis

Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS 19. Significance was measured using Fisher's least significant for the exact P values and significant differences are noted in the results. Differences with $P < 0.05$ were considered significant.

III. RESULTS

Figure II represents viability of MCF7 cells in response to different doses of *Rhamnus frangula* extract.

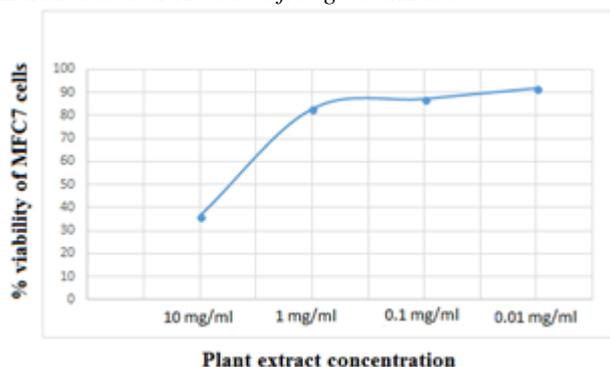


Fig 2. Viability of MCF7 cells in response to different doses of *Rhamnus frangula* extract.

The results showed that the administration of different doses (0.01g/ml, 0.1mg/ml, 1mg/ml and 10mg/ml) of *Rhamnus frangula* resulted in decreased viability of MCF7 cells ($P < 0.05$). The higher dose of extract was administered, the lower viability in MCF7 cells was observed.

IV. DISCUSSION

In our study, we reported inhibitory effect of *Rhamnus frangula* extract on cell viability of MCF7 (breast cancer) cells. Our findings also indicated that higher doses of *Rhamnus frangula* extract had higher cytotoxic effects on MCF7 cells. In line with our study there are other reports indicating that administration of some plant extracts inhibit tumor growth including breast tumors. It has also been shown that there are other plant extracts acting as anticancer against breast cancer cell [13], [14].

According to previous studies that indicate the presence of effective compound, emodin, in *Rhamnus frangula*, it seems that the cytotoxic effects of *Rhamnus frangula* result from emodin [12].

Emodin (1, 3, 8-trihydroxy-6-methylanthraquinone) is a naturally occurring anthraquinone present in the roots and barks of numerous plants, molds, and lichens, and an active ingredient of various Chinese herbs. Its inhibitory effect on mammalian cell cycle modulation in specific oncogene overexpressed cells formed the basis of using this compound as an anticancer agent. Its additional inhibitory effects on angiogenic and metastasis regulatory processes make emodin a sensible candidate as a specific blocker of tumor-associated events. Furthermore, because of its quinone structure, emodin may interfere with electron transport process and in altering cellular redox status, which may account for its cytotoxic properties in different systems [15], [16].

V. CONCLUSION

We have shown that *Rhamnus frangula* extract has inhibitory effect on viability of MCF7 cells. This discovery of

the anticancer potential of *Rhamnus frangula* may help in the development of chemo preventive drugs and may have therapeutic effects in the treatment of breast cancer.

ACKNOWLEDGMENT

We appreciate all who helped us to exert the present study.

REFERENCES

- Rushforth, K. Trees of Britain and Europe. Collins .1999. ISBN 0-00-220013-9.
- Gucker, Corey L. Frangula alnus. Fire Effects Information System. 2008.
- Bolmgren K, Oxelman B. Generic limits in *Rhamnus L.* s.l. (Rhamnaceae) inferred from nuclear and chloroplast DNA sequence phylogenies. *Taxon*. 2004. 53: 383–390. <http://dx.doi.org/10.2307/4135616>
- Frappier B, Eckert R, Lee T. Experimental Removal of the None-indigent Shrub *Rhamnus frangula* (Glossy Buckthorn): Effects on Native Herbs and Woody Seedlings. *Northeastern Naturalist*. 2004; 11(3):333-342. [http://dx.doi.org/10.1656/1092-6194\(2004\)011\[0333:EROTNS\]2.0.CO;2](http://dx.doi.org/10.1656/1092-6194(2004)011[0333:EROTNS]2.0.CO;2)
- Manojlovic NT, Solujic S, Sukdolak S, Milosev M. Antifungal activity of *Rubia tinctorum*, *Rhamnus frangula* and *Caloplaca cerina*. *Fitoterapia*. 2005 Mar; 76(2):244-6. <http://dx.doi.org/10.1016/j.fitote.2004.12.002>
- Zarzynska JM . Two faces of TGF-beta1 in breast cancer. *Epub* 2014 May 7.
- Cornejo-Moreno BA, Uribe-Escamilla D, Salamanca-Gómez F. Breast cancer genes: looking for BRACA's lost brother. *Isr Med Assoc J*. 2014 Dec; 16(12):787-92.
- Sandeep K, Singh BB, Balwinder K, Kuldeep S, Dinesh N. *RJPBCS*. 2013 Sep; 4(3):233-251.
- Lewandowska U, Owczarek K, Szewczyk K, Podsedek A, Koziolkiewicz M, Hrabec E. Influence of polyphenol extract from evening primrose (*Oenothera paradoxa*) seeds on human prostate and breast cancer cell lines. *Postepy Hig Med Dosw (Online)*. 2014; 68(0):110-8. <http://dx.doi.org/10.5604/17322693.1088036>
- Behzad S, Pirani A, Mosaddegh M. Cytotoxic activity of some medicinal plants from hamedan district of iran. *Iran J Pharm Res*. 2014 winter; 13(Suppl):199-205.
- Jafarian A, Zolfaghari B, Shirani K. Cytotoxicity of different extracts of arial parts of *Ziziphus spina-christi* on Hela and MDA-MB-468 tumor cells. *Biomed Res*. 2014; 3:38. <http://dx.doi.org/10.4103/2277-9175.125727>
- Kupchan SM, Karim A. Tumor inhibitors. 114. Aloe emodin: antileukemic principle isolated from *Rhamnus frangula L.* *Lloydia*. 1976 Jul-Aug; 39(4):223-4.
- Wicaksono BD , Handoko YA , Arung ET, Kusuma IW, Yulia D, Pancaputra AN ,et al. Antiproliferative Effect of the Methanol Extract of Piper crocatum Ruiz & Pav Leaves on Human Breast (T47D) Cells In-vitro. *Trop J Pharm Res*, August 2009; 8 (4): 345.
- Réthy B, Zupkó I, Minorics R, Hohmann J, Ocsovszki I, Falkay G. Investigation of cytotoxic activity on human cancer cell lines of arborinine and furanoacridones isolated from *Ruta graveolens*. *Planta Med* 2007; 73:41-48. <http://dx.doi.org/10.1055/s-2006-951747>
- Srinivas G, Babykutty S, Sathiadevan PP, Srinivas P. Molecular mechanism of emodin action: transition from laxative ingredient to an antitumor agent. *Med Res Rev*. 2007 Sep; 27(5):591-608. <http://dx.doi.org/10.1002/med.20095>
- Lee HZ, Hsu SL, Liu MC, Wu CH. Effects and mechanisms of aloe-emodin on cell death in human lung squamous cell carcinoma. *Eur J Pharmacol*. 2001 Nov 23; 431(3):287-95. [http://dx.doi.org/10.1016/S0014-2999\(01\)01467-4](http://dx.doi.org/10.1016/S0014-2999(01)01467-4)