eISSN 2255-0569

ORIGINAL

Investigating the toxicity of germ of date seed on normal and cancerous cell line and P53 gene expression

Investigación de la toxicidad del germen de semilla de dátil en líneas celulares normales y cancerosas y expresión del gen P53

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Received: 28 - XII - 2022
Accepted: 25 - I - 2023

doi: 10.3306/AJHS.2023.38.03.62

Abstract

In this study, the germ of Date seed was collected by breaking a thousand date kernels. These acquired germ was powdered and dissolved in distilled water for achievement of different concentrations (3.4, 1.7, 0.85, 0.425 and 0.212 mg /ml). Cancerous (MCF-7) and normal (HFF) cell lines were treated with these concentrations for 24, 48, and 72 hours. Cell viability was assessed using MTT technique. P53 gene expression was evaluated by Real time PCR technique. Results showed that in the highest concentration of germ of Date seed (3.4mg/ml), the percentage of viable cells for cancerous cell lines was 26, 30.1, 40.1% in 24, 48, and 72 hours, respectively. In this concentration, for normal cell lines, the percentage of viable cells was 36.1, 37.1, 42.1% in 24, 48, and 72 hours, respectively. In both cell line, germ of Date seed led to increase of P53 gene expression.

Key words: Date seed, Germ, Cancer, P53 Gene expression.

Resumen

En este estudio, el germen de la semilla de dátiles se recolectó rompiendo mil semillas de dátiles. Este germen adquirido fue pulverizado y disuelto en agua destilada para lograr diferentes concentraciones (3.4, 1.7, 0.85, 0.425 y 0.212 mg/ml). Las líneas celulares cancerosas (MCF-7) y normales (HFF) se trataron con estas concentraciones durante 24, 48 y 72 horas. La viabilidad celular se evaluó utilizando la técnica MTT. La expresión del gen P53 se evaluó mediante la técnica de PCR en tiempo real. Los resultados mostraron que en la mayor concentración de germen de semilla de dátil (3,4 mg/ml), el porcentaje de células viables para líneas celulares cancerosas fue de 26, 30,1, 40,1% en 24, 48 y 72 horas, respectivamente. En esta concentración, para líneas celulares normales, el porcentaje de células viables fue 36,1, 37,1, 42,1% en 24, 48 y 72 horas, respectivamente. En ambas líneas celulares, el germen de la semilla de dátil condujo a un aumento de la expresión del gen P53.

Palabras clave: Semilla de dátil, germen, cáncer, expresión del gen P53.

Introduction

Breast cancer with high frequency among women is the most important cancer worldwide[1,2]. The survival rate for women with non-metastatic invasive breast cancer is approximately 84-90%. In recent years, incidence rates of breast cancer have increased by 0.5% per year. The average risk of a woman for developed breast cancer is 13%. This means there is a 1 in 8 chance she will develop breast cancer[3]. There are various strategies for treatment of breast cancer[4]. Development of complementary medicine can be useful for enhancement of current treatment. The use of natural products, especially with plant origin, have been developed in complementary medicine[5]. Date seeds as a discarded part of Dates, have unique medicinal properties such as antioxidant and antimicrobial properties, cholesterol reduction, protective effect against radiation, anti-diabetic effect, and etc.[6-16]. In this article, we evaluated the in vitro effect of germ of Date seed on viability and P53gene expression in normal and cancerous cell line.

Materials and Methods

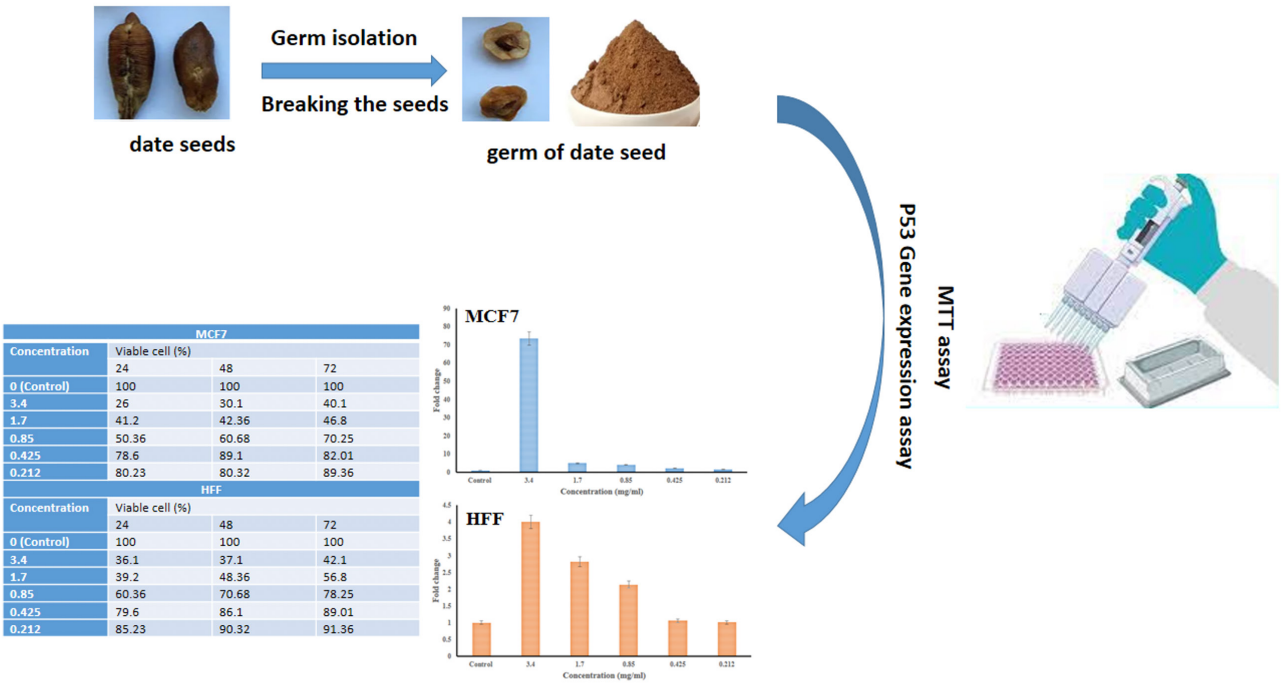
Human breast cancer and normal cell lines were used for evaluation of anticancer activity and P53 gene expression. These cells were cultured at suitable conditions. Cultures cells were treated with different concentrations of the powder of Date seed germ (4, 3, 1.7, 0.85, 0.425 and 0.212 mg /ml) for 24, 48 and 72 hours. After the treated period, the effect of powder on the cells was evaluated using MTT technique. For evaluation of P53 gene expression, Real time PCR technique was applied. In this section, the cells were cultured. After 24 hours, the powder of Date seed germ was added to the wells and placed in a CO2 incubator. RNA was extracted from these cells and cDNA was synthesized and the P53 gene expression against beta-actin gene was examined.

Results

Experimental procedures and acquired results are summarized in graphical abstract (**Figure 1**).

Experimental evaluation of germ of Date seed on normal and cancerous cells was summarized in **table I & II**. According to this table, different concentrations of germ of Date seed reduced the number of viable cells compared to the control group. The percentage of living cells decreased along with increases of concentration of powder. The toxicity of the powder on cancer cells was higher than normal cell line. The highest toxicity was observed for both cells at the highest concentration of powder. Increasing the duration of cell treatment led to a greater effect of the substance on the percentage of viable cells.

Figure 1: Graphical abstract containing experimental and result sections.



MCF7			
Concentration	Viable cell (%)		
	24	48	72
0 (Control)	100	100	100
3.4	26	30.1	40.1
1.7	41.2	42.36	46.8
0.85	50.36	60.68	70.25
0.425	78.6	89.1	82.01
0.212	80.23	80.32	89.36
HFF			
Concentration	Viable cell (%)		
	24	48	72
0 (Control)	100	100	100
3.4	36.1	37.1	42.1
1.7	39.2	48.36	56.8
0.85	60.36	70.68	78.25
0.425	79.6	86.1	89.01
0.212	85.23	90.32	91.36

Table I: Effect of different concentrations of germ powder of Date seed on cancer cells (MCF7).

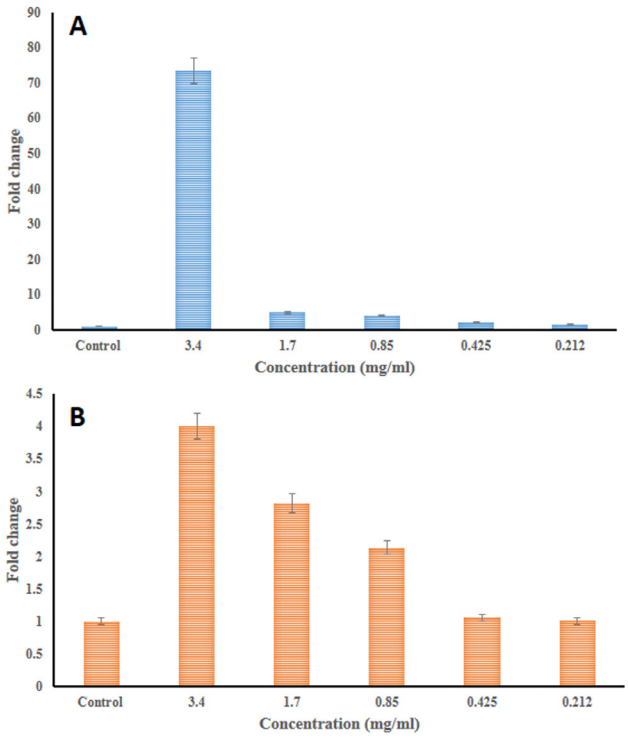
Concentration	Viable cell (%)		
	24	48	72
0 (Control)	100	100	100
3.4	26	30.1	40.1
1.7	41.2	42.36	46.8
0.85	50.36	60.68	70.25
0.425	78.6	89.1	82.01
0.212	80.23	80.32	89.36

Table II: Effect of different concentrations of germ powder of Date seed on normal cells (HFF).

Concentration	Viable cell (%)		
	24	48	72
0 (Control)	100	100	100
3.4	36.1	37.1	42.1
1.7	39.2	48.36	56.8
0.85	60.36	70.68	78.25
0.425	79.6	86.1	89.01
0.212	85.23	90.32	91.36

Figure 2 shows the results of increased P53 gene expression in both normal and cancer cell lines compared to the control group. This increase in gene expression is directly related to the concentration of powder. According to the results, the rate of increased gene expression in cancer cells was higher than normal cells. The highest concentration of powder (3.4 mg / ml) had the greatest effect on the P53 gene expression. This effect was observed in both normal and cancer cells.

Figure 1: Effect of different concentrations of germ powder of Date seed on P53 gene expression in cancer (A) and normal (B) cells.



Discussion

The p53 gene is considered as the most common and major gene that mutates in various tumors[17]. This gene, as an essential marker, plays an important role in the clinical diagnosis of tumors[18]. The researchers first identified the P53 gene as a type of cancer protein antigen, then as a cancer gene and finally as a tumor-inhibiting gene[19]. Research has shown that the wild-type p53 gene is a tumor-inhibiting gene, and mutations in the p53 gene can lead to tumorigenesis[20]. Therefore, any combination that can alter the expression of this gene can be considered as one of the therapeutic goals in the field of cancer[21-23]. Meanwhile, research on natural materials and compounds is gaining more and more attention. Date seeds are a discarded part of Dates. But the results of various studies have shown that these Dates have unique medicinal properties[6-9]. Analysis of the constituents of Date seed has shown that this part of Dates contains more than 80% carbohydrates, about 15% oils and approximately 5% protein[10]. There are also various nutrients in Date seeds. The antioxidant and antimicrobial properties of Date seed powder have been proven in various studies[11-13]. Effect of Date seed powder on cholesterol reduction in mice, effect on blood and biochemical parameters and some fertility indices, protective effect against radiation, especially gamma radiation, anti-diabetic effect, protective effect on liver and kidney function, efficacy in cerebral ischemia and etc. were proven in various studies[14-16]. Acquired powders from whole Date seed have been used in various articles and acceptable results have been obtained in terms of biological effects, but no studies have been performed on germ of Date seed. In this study, the anti-cancer effect of germ powder and its related p53 gene expression was evaluated on breast cancer cell line. The results showed that the germ powder led to cell death in both normal and cancer cells, but the toxicity of the powder was higher on cancer cells. Based on the obtained results, the toxicity effect of germ powder is dose-dependent and the survival rate of cancer cells was significantly reduced at concentrations higher than 3 mg/ml. The results of the gene expression study also showed that the extract, increased P53 gene expression was occurred in both normal and cancer cells compared with the control. The effect of germ powder on increasing gene expression was greater in cancer cells than normal cells.

Conflict of interest

None

References

1. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biological research. 2017;50(1):1-23.

2. McKinney SM, Sieniek M, Godbole V, Godwin J, Antropova N, Ashrafian H, et al. International evaluation of an AI system for breast cancer screening. Nature. 2020;577(7788):89-94.

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: a cancer journal for clinicians. 2020;70(1):7-30.

4. Waks AG, Winer EP. Breast cancer treatment: a review. Jama. 2019;321(3):288-300.

5. Mokarramat-Yazdi A, Soltaninejad H, Zare-Zardini H, Shishehbor F, Alemi A, Fesahat F, et al. Investigating the anticancer effect of a new drug originating from plant and animal: In vitro and in vivo study. Journal of Advanced Pharmacy Education & Research| Apr-Jun. 2020;10(S2):73.

6. Maqsood S, Adiamo O, Ahmad M, Mudgil P. Bioactive compounds from date fruit and seed as potential nutraceutical and functional food ingredients. Food chemistry. 2020;308:125522.

7. Mrabet A, Jiménez-Araujo A, Guillén-Bejarano R, Rodríguez-Arcos R, Sindic M. Date seeds: A promising source of oil with functional properties. Foods. 2020;9(6):787.

8. Roslan NS, Rahim NS, Razali Z, Zulkifli MA. Anti-microbial Properties and Toxicity Test of Stingless Bee Honey (Trigona itama), Ajwa Date (Phoenix dactylifera L.) Seeds and Their Combinations. Charting the Sustainable Future of ASEAN in Science and Technology: Springer; 2020. p. 155-66.

9. Abdulrahman LNMA. Impacts of Date Palm Seeds (Phoenix Dactyliferous L.) on Common Carpcyprinus Carpio L. Biological Indices and Blood Pictures. EXECUTIVE EDITOR. 2020;11(01):1910.

10. Ataye SE, Hadad KM, Lame S, Habibi NM, Fatemi S. Determination of chemical composition and fatty acids profile of date seed. 2011.

11. Saryono S, editor Date seeds drinking as antidiabetic: a systematic review. IOP Conference Series: Earth and Environmental Science; 2019: IOP Publishing.

12. Metoui M, Essid A, Bouzoumita A, Ferchichi A. Chemical Composition, Antioxidant and Antibacterial Activity of Tunisian Date Palm Seed. Polish Journal of Environmental Studies. 2019;28(1).

13. Ardekani MRS, Khanavi M, Hajimahmoodi M, Jahangiri M, Hadjiakhoondi A. Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran. Iranian journal of pharmaceutical research: IJPR. 2010;9(2):141.

14. Abou-Zeid SM, El-Bialy BE, El-Borai NB, AbuBakr HO, Elhadary AMA. Radioprotective effect of date syrup on radiation-induced damage in rats. Scientific reports. 2018;8(1):1-10.

15. Habib H, Othman A, Al-Marzooqi S, Al-Bawardi A, Pathan JY, Hilary S, et al. The antioxidant activity of date seed: preliminary results of a preclinical in vivo study. Emirates Journal of Food and Agriculture. 2017:822-32.

16. Saryono S, Proverawati A. Hepatoprotective effect of date seeds works through the antioxidant mechanism: a systematic review. Annals of Tropical Medicine and Health. 2019;22:301-9.

17. Ma Y-S, Shi Y, Liu J-B, Wu T-M, Jia C-Y, Yang H-Q, et al. Targeting long non-coding RNA to therapeutically regulate gene expression in cancer. Molecular Therapy-Nucleic Acids. 2020.

18. Puetkasichonpasutha J, Namwat N, Sa-Ngiamwibool P, Titapun A, Suthiphongchai T. Evaluation of p53 and Its Target Gene Expression as Potential Biomarkers of Cholangiocarcinoma in Thai Patients. Asian Pacific journal of cancer prevention: APJCP. 2020;21(3):791.

19. Graziano SL, Tatum A, Herndon II JE, Box J, Memoli V, Green MR, et al. Use of neuroendocrine markers, p53, and HER2 to predict response to chemotherapy in patients with stage III non-small cell lung cancer: a Cancer and Leukemia Group B study. Lung Cancer. 2001;33(2-3):115-23.

20. Maniwa Y, Yoshimura M, Obayashi C, Inaba M, Kiyooka K, Kanki M, et al. Association of p53 gene mutation and telomerase activity in resectable non-small cell lung cancer. Chest. 2001;120(2):589-94.

21. Tamura RE, Lana MG, Costanzi-Strauss E, Strauss BE. Combination of cabazitaxel and p53 gene therapy abolishes prostate carcinoma tumor growth. Gene therapy. 2020;27(1):15-26.

22. Roshankhah S, Arji Rodsari B, Jalili C, Salahshoor MR. The Role of Harmine in Up-regulating p53 Gene Expression and Inducing Apoptosis in MCF-7 Cell Line. Middle East Journal of Cancer. 2020;11(1):34-41.

23. Duffy MJ, Synnott NC, O'Grady S, Crown J, editors. Targeting p53 for the treatment of cancer. Seminars in Cancer Biology; 2020: Elsevier.