Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Mini Review

Ziziphus Jujube: a review study of its anticancer effects in various tumor models *invitro* and *invivo*

S. Ebrahimi[1], H. Mollaei[2], R. Hoshyar[2*]

[1] Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran
[2] Cellular & Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

Correspondence to: hooshyar@bums.ac.ir

Received May 8, 2017; **Accepted** October 25, 2017; **Published** October 31, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.10.19

Abstract: The growing rate of cancer incidences and inefficiency of current therapies have made scientists to find novel agents such as medicinal plants. *Ziziphus jujube* is one of these plants whose pharmaceutical effects have been studied for a long time. Recent investigations have proved that bioactive compounds of *Ziziphus jujube* including triterpenic acids, flavonoids, cerebrosides, phenolic acids, α tocopherol, β carotene, and polysaccharides have anti-proliferative effects on several cancerous cell lines and animals. The present study aimed to cover all previous *invitro* and *invivo* findings and some of molecular mechanisms of its anticancer property.

Key words: *Ziziphus jujube*; Cancer; *Invivo*; *Invitro*; Molecular mechanism; IC50.

Introduction

During the past decades, increasing incidence of cancer has turned into a serious health problem all over the world (1). Despite many advantages of modern medicine, it is not completely effective. Hence, scientists have attempted new compounds as complementary or alternative therapies. Natural products have always been a major source of drugs for various diseases such as cancer (2). The National Cancer Institute (NCI) of the United States screened over 35,000 plant extracts in the 1960s and this valuable investigation led to the discovery of important anticancer compounds such as camptothecin, taxol and vinblastine, which are still used clinically (3).

Ziziphus jujube is a functional food with nutritional value belonging to *Rhamnaceae* family. It has two main species including *Ziziphus mauritiana* Lam. and *Ziziphu sjujube* Mill (4). Several studies have reported jujube's effect on various diseases such as diabetes, diarrhea, Alzheimer, anxiety, fungal disease, hepatic disease, skin infections, liver complaints, urinary disorders, obesity, fever, pharyngitis, bronchitis, anemia, insomnia, gastrointestinal tract disorders and cancer (4, 5). The active compounds of *Ziziphus* jujube, which are responsible for the mentioned effects, are triterpenic acids, flavonoids, cerebrosides, phenolic acids, αtocopherol, βcarotene, and polysaccharides (6). In the present paper, we aimed to briefly review the anticancer effects of *Ziziphus jujube invitro* and *invivo*.

Anticancer activity of *Ziziphus jujube invitro*

Many studies have focused on anticancer effects of *Ziziphus jujube* extract or its active compounds in different cancerous cell lines as summarized in Table 1. This inhibitory effect might be due to induction of apoptosis and cell cycle arrest via changing the expression level of the related genes (Table 1). On the other hand, as shown in Table 2, the cytotoxicity potential of various components of *Ziziphus jujube* is strongly different. Thus, further investigations are needed to reveal the exact molecular mechanisms of these effects (6).

Anticancer activity of *Ziziphus jujube invivo*

In addition to *invitro* investigations, various studies have shown the anticancer activity of *Ziziphus jujube invivo*. As a traditional medicine, CKBM is a combination of yeast and herbs including *Ziziphus jujube*. Chan et al and Shin et al in 2004 demonstrated the anti-tumorigenic and pro-apoptotic effects of CKBM on human hepatocellular carcinoma and gastric cancer in nude mice, respectively (7, 8). Moreover, several studies have reported that combination of four herbs *Scutellariabaicalensis Geori*, *Glycyrrhizauralensis Fisch*, *Paeonialactiflora Pall*, and *Ziziphus jujube Mill,* known as PHY906, has a significant anticancer effect and increases the cytotoxicity of chemotherapeutic agents such as CPT-11, Irinotcan and Capecitabin in female BDF-1 mice suffering from subcutaneous Colon tumor, colorectal cancer and hepatocellular carcinoma respectively (9-11). Additionally, it has been shown that bioactive compounds of *Ziziphus jujube* such as betulinic acid and ursolic acid have anti-tumorigenic and pro-apoptotic effects *invivo* (12, 13).

In another study in 2014, antitumor mechanism of *Jujuboside B* was investigated *invivo* and *invitro*. The results indicated that *Jujuboside B* inhibited tumor growth in a tumor xerograph model bearing HCT 116 cells.

Table 1. Anti-cancer effects of Ziziphus Jujube *invitro.*

Ref	Cellular/ molecular effect	IC50	Unit	Positive control	Method	Cell line(s)	Ziziphus extract/ compound
(15)	Inhibition of cell growth in a dose- and time-dependent manner	1.8, 1 and 0.5 after 24, 48, and 72 h respectively	mg/mL	___	MTT	MDA-MB-468	Fruit of Ziziphus jujube aqueous extract
(21)	Inhibition of cell growth and induction of apoptosis	1.8, 1 and 0.5 after 24, 48, and 72 h respectively	mg/mL	___	MTT	MCF-7	Fruit of Ziziphus jujube aqueous extract
	Inhibition of cell growth	1.5, 0.5 and 0.2 after 24, 48, and 72 h respectively				OV2008	
(26)	Inhibition of cell growth via up-regulating the expression of P53, P21 and P27 as well as down-regulating cyclin D1 expression	1.2, 0.5 and 0.2 after 24, 48, and 72 h respectively	mg/ml	___	MTT	OV2008	Fruit of Ziziphus jujube aqueous extract
(3)	High antioxidant activity and induction of apoptosis	___	mg/ mL	Ceramide	MTT	Caco-2	Fruit of Ziziphus Mucronata Dichloromethane extract
							Fruit of Ziziphus Mucronata Ethanol extract
							Fruit of Ziziphus Mucronata Methanol extract
		2.5 after 24h				Hela	Fruit of Ziziphus Mucronata Ethanol extract
							Fruit of Ziziphus Mucronata Dichloromethane extract
(29)	Anti-proliferation capability in a dose- and time-dependent course; cell cycle arrest at the G2/M phase; induction of apoptosis; and increased caspase-3 and caspase-9 activity	3.99 and 3.36 after 24, 48 h respectively	mg/mL	5Flurouracil	MTT	melanoma cancer cell line	Deproteinized polysaccharide (DPP) isolated from fruit of Ziziphus jujube
(4)	Inhibition of cell growth and induction of apoptosis	401.6,312.0 and 232.4 after 24, 48 and 72 h respectively	µg/ml	Melphalan	Neutral Red Uptake	Jurkat	ethanolic extracts from seeds of three jujube cultivars (Rianthong, Taiwan, and Jumbo)

(22)	Inhibition of cell growth	25.32 after 72 h	µM	Paclitaxel	MTT	MCF-7	Betulinic acid (BA) isolated from sour ziziphus jujube fruit
(25)	Inhibition of cell growth	___	___	Doxorubicin	MTT	MDA-MB-468	different extracts of leaves of Ziziphus spina-christi
						Hela	
(20)	Inhibition of cell growth and induction of apoptosis	0.1, 10 and 20 after 72h.	µg/mL	___	MTT	HEp-2	water extract of dried fruit of Ziziphus Jujube
						HeLa	
						Jurkat	
(23)	Inhibition of cell growth and induction of apoptosis	14.42, 7.64, 1.69 for ZE1, ZE2, and ZE4 respectively.	µg/mL	___	MTT	MCF-7	Fruite of lyophilized Ziziphus.j n-hexane extract (ZE1)
							chloroform extract (ZE2)
		14.06, 6.21, 3.70 for ZE1, ZE2, and ZE4 respectively				SKBR3	methanol extract (ZE4)
(24)	Inhibition of cell growth	20.71 and 11.24 for EE and IC respectively	µg/mL	Tamoxifen	MTT	MCF-7	Ethanolic extract (EE) and isolated identified compound (IC) from Ziziphus nummularia root bark
		19.21 and 11.17 for EE and IC respectively				K-562	
		15.35 and 11.51 for EE and IC respectively				OVCAR-3	
		26.42 and 14.65 for EE and IC respectively				HT-29	
		24.45 and 15.27 for EE and IC respectively				A-498	
(31)	Inhibition of cell growth and induction of apoptosis Cell cycle arrest	20	mg/ml	___	Neutral Red Uptake	HepG2	Fruit of Ziziphus jujube
(14)	Inhibition of cell growth and induction of apoptosis	114 after 24h	µM	___	MTT	HCT116	Jujuboside B isolated from the seeds of Ziziphus jujube
		107 after 24h				AGC	
(28)	Inhibition of cell growth	>100	µg/mL	___	MTT	HeLa, A549, U937	Methanol extract from fruit of Ziziphus jujube
(30)	Inhibition of cell growth	Table 2	µg/mL	___	MTT	MGC-803, HT-29, NCI-H460, HepG-2	six isolated compound from Ziziphus jujube fuite

(27)	Inhibition of cell growth	20 after 48h 40 after 48h 40 after 48h	µg/mL	___	MTT	HL-60 Molt-4 HeLa	aqueous-ethanolic seed extract of Ziziphus Jujube
(32)	Inhibition of cell growth and induction of caspase-dependent apoptosis	417.7 after 24 h 487.9 after 24 h 232.4 after 24 h 333.4 after 24 h 312.0 after 24 h 401.6 after 24 h	µg/mL	Melphalan	Modified Neutral Red Assay	Jurkat	Ziziphus jujube Samros seed ethanolic extract (SEE) Z.j Bombay SEE Z.j Taiwan SEE Z.j Nomsod SEE Z.j Jumbo SEE Z.j Rianthong SEE
(33)	Inhibition of cell growth	almost >20 µM	µM	Adriamicyne	Sulforhodamin B (SRB)	K562 B16(F10) SK-MEL-2 PC-3 LOX-IMVI A549	Eleven triterpenoic acids isolated from Ziziphus jujube fruit

Table 2. The inhibition of compounds against the proliferation of MGC-803, HT-29, NCI-H460 and HepG-2 tumor cell lines.

Compound	IC50, µg/mL			
	MGC-803	HT-29	NCI-H460	HepG-2
zizyberenalic acid	13.92	12.67	20.67	23.32
ceanothenic acid	48.60	>100	96.32	67.36
betulinic acid	>100	40.23	>100	>100
oleanolic acid	35.34	>100	34.41	>100
ceanothic acid	95.33	>100	>100	81.27
epiceanothic acid	>100	>100	98.96	>100

Also, molecular studies have showed that this treatment reduces the expression of the proliferation biomarker Ki-67 in tumor tissues (14). In the same year, Hoshyar et al reported that the *Ziziphus jujube* has anti-cancer effect on mammary tumors. This effect could be due to anti-oxidant properties of *Z. jujube* compound. Moreover, the results showed that among different biochemical parameters, serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels, total protein, and albumin were significantly changed by *Ziziphus jujube* treatment (15).

Liu et.al in 2015 investigated the protective effects of dietary *Ziziphus jujube* on colitis-associated colon carcinogenesis in azoxymethane (AOM)-dextran sodium sulphate (DSS)-treated mice. The results showed that this treatment led to aberrant crypt foci (ACF) formation and declined progression of hyperplasia to dysplasia (16).

Discussion

Cancer is one of the main leading causes of death worldwide. Current therapies such as surgery, radiotherapy and chemotherapy are partly effective at the first steps. However, over time, resistance of tumor cells leads to recurrence of disease symptoms. Moreover, unwanted side effects of these therapies remain as another challenge to the oncologist (17).

Pharmacological properties of plant-derived natural products such as flavonoids, terpenes, alkaloids, etc. have received considerable attention in recent decades. Hence, researchers try to use them as a valuable source of complementary medicine (18, 19).

Ziziphus jujube is a medicinal plant in the Iranian traditional medicine and is defined as a laxative and blood purifier. It is believed that the dried fruits of *Ziziphus Jujube* have anticancer, anodyne, pectoral, refrigerant, sedative, stomachic, styptic and tonic properties and acts as immune response enhancer (6, 20).

Several studies have shown that *Ziziphus Jujube* has anti-proliferative and apoptotic effects on different cancerous cell lines such as breast (15, 21-25), cervix (3, 20, 21, 25-28), ovary (24), melanoma (29), leukemia (24, 27), lymphoma (4, 20, 27, 28, 30), hepatocellular carcinoma (20, 31), gastric (14, 30), colorectal (3, 14, 24, 30), kidney (24) and lung (28, 30). On the other hand, *invivo* studies have proved these effects on hepatocellular carcinoma (11), breast (15), gastric and colon (10, 14, 16) cancers. These anti-cancer effects might be due to alternation of gene expression such as up-regulation of different caspases, P53, P21 and P27 and also, down-regu lation of cyclin D1 (As shown in table 1) that leads to inducing apoptosis or cell cycle arrest. However as there are lots of cancer related mechanisms, it would be possible that *Ziziphus Jujube* affect various regulators in cancer related signaling pathways. Also, non-coding

regulators such as microRNAs and long non-coding RNAs (lncRNAs) might be as one of the important factors that modulate apoptosis and proliferation of cancerous cells. So, studding the effect of *Ziziphus Jujube* on these factors will be valuable. Altogether, more studies need to better understanding the molecular mechanism of *Ziziphus Jujube* and its major components action. These complementary studies are essential to design novel therapeutic approaches that target main pathways and increase the efficiency of common therapies as well as other herb, Saffron (34).

Interest conflict

The authors declare no conflict of interest.

Author's contribution

SE and HM collected information and written a first draft of manuscript. RH revised and completed the review.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians. 2015;65(1):5-29.
2. Kaur R, Kapoor K, Kaur H. Plants as a source of anticancer agents. J Nat Prod Plant Resour. 2011;1(1):119-24.
3. Ilonga SK. Anticancer, antioxidant and antimicrobial screening of extracts from Ziziphus Mucronata, heliotropium ciliatum and Gnidia polycephala from the Oshikoto region of Namibia: Department of Molecular Biology and Biotechnology, University of Dar es Salaam; 2012.
4. Taechakulwanijya N, Weerapreeyakul N, Barusrux S, Siriamornpun S. Apoptosis induction effect of three jujube cultivars in HepG2 and Jurkat cell lines. International Journal of Bioscience, Biochemistry and Bioinformatics. 2013;3(6):540.
5. Tahergorabi Z, Abedini MR, Mitra M, Fard MH, Beydokhti H. "Ziziphus jujuba": A red fruit with promising anticancer activities. Pharmacognosy reviews. 2015;9(18):99.
6. Gao Q-H, Wu C-S, Wang M. The jujube (Ziziphus jujuba Mill.) fruit: a review of current knowledge of fruit composition and health benefits. Journal of agricultural and food chemistry. 2013;61(14):3351-63.
7. Chan J, Cheung J, Luk S, Wu Y, Pang S, Fung K. Anti-Cancer and Pro-Apoptotic Effects of an Herbal Medicine and Saccharomyces Cerevisiae Product (CKBM) on Human Hepatocellular Carcinoma HepG2 Cells Invitro and Invivo. Immunopharmacology and immunotoxicology. 2004;26(4):597-609.
8. Shin VY, So WH-L, Liu ES-L, Wu Y-J, Pang S-F, Cho C-H. Antitumorigenic and Pro-apoptotic effects of CKBM on gastric cancer growth in nude mice. 2004.
9. Eng C. Are herbal medicines ripe for the cancer clinic? Science Translational Medicine. 2010;2(45):45ps1-ps1.
10. Wang E, Bussom S, Chen J, Quinn C, Bedognetti D, Lam W, et al. Interaction of a traditional Chinese Medicine (PHY906) and CPT-11 on the inflammatory process in the tumor microenvironment. BMC medical genomics. 2011;4(1):38.
11. Yen Y, So S, Rose M, Saif MW, Chu E, Liu S-H, et al. Phase I/II study of PHY906/capecitabine in advanced hepatocellular carcinoma. Anticancer research. 2009;29(10):4083-92.
12. Damle AA, Pawar YP, Narkar AA. Anticancer activity of betulinic acid on MCF-7 tumors in nude mice. 2013.
13. De Angel RE, Smith SM, Glickman RD, Perkins SN, Hursting SD. Antitumor effects of ursolic acid in a mouse model of postmenopausal breast cancer. Nutrition and cancer. 2010;62(8):1074-86.
14. Xu M-Y, Lee SY, Kang SS, Kim YS. Antitumor activity of jujuboside B and the underlying mechanism via induction of apoptosis and autophagy. Journal of natural products. 2014;77(2):370-6.
15. Hoshyar R, Mohaghegh Z, Torabi N, Abolghasemi A. Antitumor Activity of Aqueous Extract of Ziziphus Jujube Fruit in Breast Cancer: An Invitro and Invivo Study. Asian Pacific Journal of Reproduction. 2015;4(2):116-22.
16. Periasamy S, Liu C-T, Wu W-H, Chien S-P, Liu M-Y. Dietary Ziziphus jujuba Fruit Influence on Aberrant Crypt Formation and Blood Cells in Colitis-Associated Colorectal Cancer in Mice. Asian Pacific Journal of Cancer Prevention. 2015;16(17):7561-6.
17. Chabner BA, Roberts TG. Chemotherapy and the war on cancer. Nature Reviews Cancer. 2005;5(1):65-72.
18. Manju K, Jat R, Anju G. A review on medicinal plants used as a source of anticancer agents. International Journal of Drug Research and Technology. 2017;2(2):6.
19. Goyal S, Gupta N, Chatterjee S, Nimesh S. Natural Plant Extracts as Potential Therapeutic Agents for the Treatment of Cancer. Current topics in medicinal chemistry. 2017;17(2):96-106.
20. Vahedi F, Najafi MF, Bozari K. Evaluation of inhibitory effect and apoptosis induction of Zyzyphus Jujube on tumor cell lines, an invitro preliminary study. Cytotechnology. 2008;56(2):105-11.
21. Abedini MR, Erfanian N, Nazem H, Jamali S, Hoshyar R. Anti-proliferative and apoptotic effects of Ziziphus Jujube on cervical and breast cancer cells. Avicenna Journal of Phytomedicine. 2016;6(2):142.
22. Sun Y-F, Song C-K, Viernstein H, Unger F, Liang Z-S. Apoptosis of human breast cancer cells induced by microencapsulated betulinic acid from sour jujube fruits through the mitochondria transduction pathway. Food chemistry. 2013;138(2):1998-2007.
23. Plastina P, Bonofiglio D, Vizza D, Fazio A, Rovito D, Giordano C, et al. Identification of bioactive constituents of Ziziphus jujube fruit extracts exerting antiproliferative and apoptotic effects in human breast cancer cells. Journal of ethnopharmacology. 2012;140(2):325-32.
24. Dey Ray S, Dewanjee S. Isolation of a new triterpene derivative and invitro and invivo anticancer activity of ethanolic extract from root bark of Zizyphus nummularia Aubrev. Natural product research. 2015;29(16):1529-36.
25. 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. Advanced biomedical research. 2014;3(1):38.
26. Hoshyar R, Jamali S, Fereidouni M, Abedini M. The cytotoxic activity of Ziziphus Jujube on cervical cancer cells: Invitro study. Cellular and molecular biology (Noisy-le-Grand, France). 2014;61(8):128-30.
27. Mishra T, Khullar M, Bhatia A. Anticancer potential of aqueous ethanol seed extract of Ziziphus mauritiana against cancer cell lines and Ehrlich ascites carcinoma. Evidence-Based Complementary and Alternative Medicine. 2010;2011.
28. Choi S-H, Ahn J-B, Kim H-J, Im N-K, Kozukue N, Levin CE, et al. Changes in free amino acid, protein, and flavonoid content in jujube (Ziziphus jujube) fruit during eight stages of growth and antioxidative and cancer cell inhibitory effects by extracts. Journal of agricultural and food chemistry. 2012;60(41):10245-55.
29. Hung C-F, Hsu B-Y, Chang S-C, Chen B-H. Antiproliferation of melanoma cells by polysaccharide isolated from Zizyphus jujuba. Nutrition. 2012;28(1):98-105.
30. Guo S, Duan J-a, Tang YP, Su SL, Qian DW. Triterpenoid acids from Ziziphus jujuba. Chemistry of Natural Compounds. 2011;47(1):138-9.
31. Huang X. Mechanism of the growth inhibitory effects of Zizyphus jujuba and green tea extracts in human hepatoma cells: Thesis, Department of Food and Human Health Sciences, Graduate

School of Human Life Science, Osaka City University, Japan; 2008.
32. Taechakulwanijya N, Weerapreeyakul N, Barusrux S, Siriamornpun S. Apoptosis-inducing effects of jujube (Zao) seed extracts on human Jurkat leukemia T cells. Chinese medicine. 2016;11:15.
33. Lee SM, Min BS, Lee CG, Kim KS, Kho YH. Cytotoxic triterpenoids from the fruits of Zizyphus jujuba. Planta medica. 2003 Nov;69(11):1051-4.
34. Hoshyar R, Khayati GR, Poorgholami M, Kheykhaii M. A novel green one-step synthesis of gold nanoparticles using crocin and their anti cancer activities. Journal of Photochemistry and photobiology, B: biology. 2016;159:237-242.