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Antitumor activity of aqueous extract of *Ziziphus jujube* fruit in breast cancer: An *in vitro* and *in vivo* study

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ABSTRACT

Objective: To investigate various therapeutic effects of medicinal plant *Ziziphus jujube* (*Z. jujube*), such as antibacterial, antiviral, anticancer, anti-inflammatory and antioxidant properties. **Methods:** The present study was conducted to assess the beneficial effects of aqueous extract of *Z. jujube* fruit on the proliferation of breast cancer cells by MTT assay and the antioxidant by FRAP, haematological and biochemical alterations caused by NMU-induced mammary carcinogenesis in rats. The rats were divided into five groups, control group A (n=10), Z. jujube control group B (n=10), NMU control group C (n=15), *Z. jujube* treatment group D (n=15) and *Z. jujube* prevention group E (n=15). At the end of the experimental period, all the animals were euthanized and blood was collected by heart puncture. **Results:** The *Z. jujube* revealed a dose- and time-dependent cytotoxic effect against an MDA-MB-468 cell line and its treatment significantly (P<0.05) increased the total antioxidant capacity when compared to the NMU control group C. *Z. jujube* exhibited a preventive effect against anaemia, lymphocytosis and neutrophilia in group D and group E, when compared to group C. Biochemical analyses showed normal levels of enzymes of the liver in the *Z. jujube* treated groups: B, D and E rats, whereas NMU control group C showed significant (P<0.05) decreases in ALT, AST, albumin and total protein levels, and significant (P<0.05) increases in ALP and LDH. **Conclusions:** These findings indicate that the *Z. jujube* ameliorates the adverse effects of NMU carcinogenesis and could be useful for treating mammary tumours in humans.

1. Introduction

Breast cancer is a complex and heterogeneous disease with respect to pathology, biochemistry and aetiology, and is the second leading cause of can-cer death. In economically developed and developing countries, breast cancer in females is the most common diagnosed cancer[1, 2]. Treatments for breast cancer usually require a multimodal approach including medical, surgical and radiological treatments to reduce mortality and improve palliation in women where cure is not a possibility. Current medical treatments in breast cancer consist of chemotherapy, hormonal treatment and targeted therapy, which are sophisticated, expensive, relatively ineffective and not widely available. Although 70 % of breast cancers are oestrogen-dependent, its aetiology remains obscure and primary prevention strategies are not yet available[3, 4]. Therefore, the development of novel anticancer agents with fewer side effects derived from natural products, especially plants, may provide an alternative and cost-effective treatment modality.

Plants have been demonstrated to be a source of clinically relevant anticancer compounds[5]. Common jujube (*Ziziphus jujube*), a plant of the Rhamnaceae family[6], is mainly distributed in the regions of Asia such as Iran, China and India[7]. The mature fruit is red to

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purplish-black and wrinkled, looking like a small date. The fruit has a single hard seed, similar to an olive stone. It is used as both a delicious fruit and an effective herbal remedy with therapeutic effects in various diseases such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, pharyngitis, bronchitis, anaemia, diarrhoea, insomnia and cancer[8, 9]. Much effort has been devoted to verifying the effectiveness of *Z. jujube* against cancer. Indeed, *Z. jujube* extracts, alone or in combination with other botanical formulations, have been shown to exert anticancer effects on several tumour cell lines in animal models of cancer in previous studies[10–17]. However, to the best of our knowledge, the anti-proliferative or cytotoxic effects of Ziziphus extracts on breast cancer cells have not yet been reported.

The aim of the present study was to investigate the anticancer activity of *Z. jujube*, a natural product, in both *in vitro* (MDA-MB-468 cells as human breast cancer cells) and *in vivo* (N-Methyl-N-Nitrosourea or NMU- induced breast cancer in rats) models. To achieve this aim, the following studies were conducted into its inhibitory effect on the proliferation of breast cancer cell line MDA-MB-468, and some of the biological effects of *Z. jujube* extract on the antioxidant, haematological and biochemical alterations caused by chemically-induced mammary carcinogenesis in Wistar rats.

2. Materials and methods

2.1. Plant extraction

The semi-dried fruits of *Z. jujube* were washed and then their seed were separated from soft red parts and removed. The samples were dried in 50 ℃ and were grounded into powder in a mortar. The powder dissolved in boiling distilled water for 30 minute. The mixture was filtered and then samples lyophilized. The dried aqueous extracts were later diluted in RPMI medium and prepared at different concentrations.

2.2. In vitro cytotoxicity assay

MDA-MB-468 cell line was provided kindly from Iranian Biological Resource Center (IBRC), Tehran, Iran. The cells were cultured in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum, 100 units/mL penicillin and 100 mg/mL streptomycin. The cells were grown at 37 ℃ in a humidified atmosphere containing 5% CO_2. The cells were treated with different concentrations of *Z. jujube* extracts (0–1200 μg/mL) at various times (24, 48 and 72 h). All treatments were performed in triplicate. Cell proliferation was determined using the 3-(4,5-dimethylthiazol- 2-yl)-2,5- diphenyl tetrazolium (MTT) assay[18]. The cells were seeded onto the 96-well plates and different concentrations of *Z. jujube* extracts were added to the cells. After incubation at different time courses, the medium was removed, and 100 μL of MTT solution (1 mg/mL in RPMI) was added to each well for 4 h. The supernatant was removed by aspiration, and 200 μL dimethyl sulfoxide (DMSO) was added to the wells to dissolve any precipitate. The absorbance was then read at 540 nm using an ELISA plate reader. The cytotoxic effect of *Z. jujube* extracts on the MDA-MB-468 cells was expressed as IC_{50} values (the drug concentration that reduced the absorbance of the treated cells by 50% compared with the control sample). The IC_{50} value of *Z. jujube* extracts was calculated using the dose- and time-dependent curves by linear interpolation. In addition, the cell viability was calculated by dividing the absorbance of treated cells in each well to the mean absorbance of control cells.

2.3. In vivo anticancer activity

In this experimental study, female Wistar Albinorats (n=65), 40 days old, were purchased from the Animal Center at Birjand University of Medical Sciences, Birjand, Iran. They were housed at five animals per cage in a room with controlled lighting (lights on from 06:00–20:00) and temperature of (23 ± 2) ℃ in the Animal House at Birjand University of Medical Sciences. The animals were fed a standard laboratory diet with access to water ad libitum. They were acclimated for about two weeks before the start of the study. The experimental protocol was approved by the Animal Ethical Committee in accordance with the Guidelines for the Care and Use of Laboratory Animals prepared by Birjand University of Medical Sciences. The animals were weighed weekly. Animals in control group (n=10) only received vehicle injections (Group-A), in *Z. jujube* control group (n=10) received vehicle injections and aqueous extraction of *Z. jujube* at the dose of 400 mg/kg body weight by gavages (Group-B), in NMU group (n=15) received only NMU (Sigma, St. Louis, MO) at the dose rate of 60 mg/kg body weight intraperitoneally (*i.p*) five times at 50, 70, 80, 90 and 110 days of age (Group-C), in *Z. jujube* treatment group (n=15) administered with NMU and after appearance of palpable tumors, treated with aqueous extraction of *Z. jujube* (400 mg/kg body weight) for 30 days (Group-D) and in *Z. jujube* prevention group (n=15) administered with NMU and *Z. jujube* (400 mg/kg body weight) administered 21 days before NMU injections (Group-E).

2.3.1. Antioxidant activity of serum

Total antioxidant capacity was measured using ferric reducing antioxidant power (FRAP) assay in rat serum that was determined by Spectrophotometer technique[19].

2.3.2. Hematology

Red Blood Cells(RBC), haemoglobin (Hb), Packed Cell Volume (PCV), total White Blood Cells(WBC), Differential Leukocyte Count (DLC)and thrombocyte count was estimated using an automated blood analyzer (Cell Dyn®3700, Abbott Dignostic, USA).

2.3.3. Serum biochemistry

Blood was collected in sterile vial without anticoagulant for serum separation. Sera samples were analyzed for biochemical parameters such as total protein, albumin, calcium, alanine amino teransferases, (ALT/SGPT), aspartate amino teransferases(AST/SGOT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) using standard commercial kits (Pars Azmoon, Tehran, Iran).

2.4. Statistical analysis

All experiments were done in triplicate, independently, and the results were expressed as the mean ± SD. Statistical analyses of the data were performed using the SPSS software, version 9.01. Results were expressed as mean ± SD of the indicated number of independent experiments. Analysis of variance (ANOVA) was used to compare the mean value of data and $P<0.05$ were considered as significant.

3. Results

3.1. In vitro cytotoxicity assay

Figure 1 shows the significant inhibitory effect of *Z. jujube* aqueous extracts on the MDA-MB-468 cell growth in a dose- and time-dependent manner ($P<0.001$). Analyses of the cell survival by MTT assay showed that the IC_{50} values of this extract on the breast cancer cells were 1.8, 1 and 0.5 mg/mL after 24, 48, and 72 hours respectively.

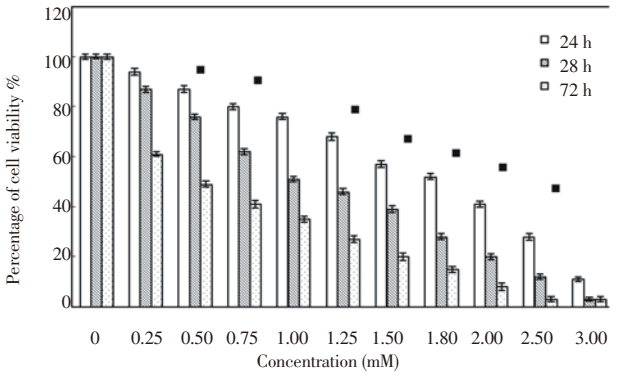

Figure 1. Effect of aqueous extract of *Z. jujube* fruit on the viability (%) of MDA-MB-468 cells.
Cells were treated with different concentrations of *Z. jujube* for 24, 48, and 72 h. Results are reported as the mean ± SD. ■: $P<0.001$ compared with control.

3.2. In vivo anticancer activity

3.3.1. Rat survival and body weight

The results revealed that no significant differences in survival were observed between groups, with approximately 97% of the animals surviving to study termination (range = 89%–99%). Figure 2 shows the changes in weight of all animals during the experiment as well as the lower weight of animals in the NMU-treated group (Group C). The mean body weights of rats receiving 400 mg/kg of *Z. jujube*(Group B) did not significantly ($P<0.05$) differ from controls (Group A) over time. However, the mean body weight of rats exposed to NMU plus 400 mg/kg of *Z. jujube* groups (groups D and E) were only 80% that of controls (Group A) by the end of the study.

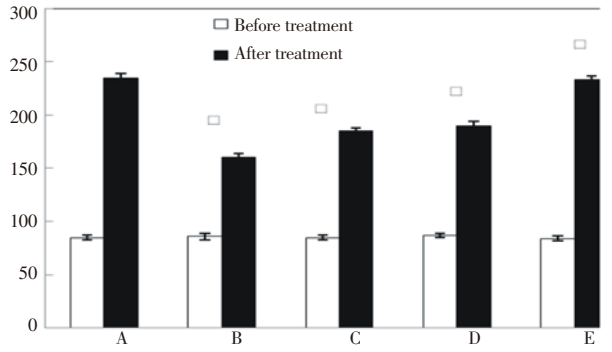

Figure 2. Effects of NMU on rats. Changes in the body weight of rats during the course of the experiment. □: $P<0.05$ compared with control. Group A: vehicle control, group B: *Z. jujube* control, Group C: NMU control, Group D: *Z. jujube* treatment and Group E: *Z. jujube* prevention.

3.3.2. FRAP assay

The changes in antioxidant power (FRAP) were shown in Figure 3. FRAP was found to be lower ($P<0.05$) in serum of NMU-treated rats compared to control ones. Antioxidant power was significantly increased in *Z. jujube* treated rats ($P<0.05$) both of treatment and prevention groups. So, treatment with aqueous extraction of *Z. jujube* improved antioxidant power.

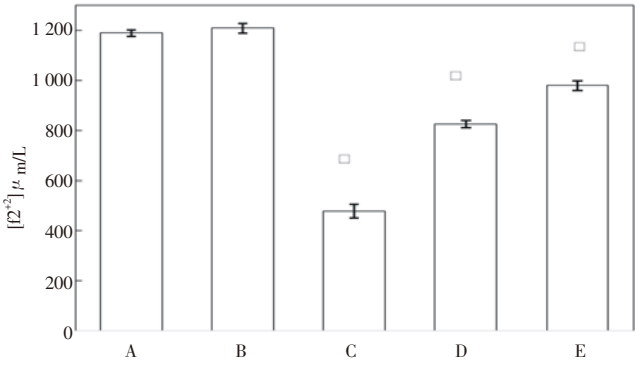

Figure 3. Antioxidant capacity of the serum of all rats at the end of experiment, which was measured by FRAP method. □: P<0.05 compared with control. Group A: vehicle control, group B: *Z. jujube* control, Group C: NMU control, Group D: *Z. jujube* treatment and Group E: *Z. jujube* prevention

3.3.3. Haematology

Our results about Red Blood Cells (RBC), hemoglobin (Hb) and Packed Cell Volume (PCV) count indicted the NMU control group-C rats exhibited significant (P<0.05) decrease in RBC, Hb and PCV levels indicating erythropaenia or anaemia when compared to other rats (Groups A,B,D and E) . *Z. jujube* treated prevention and treatment rats (Groups D and E) showed normal values of RBC, Hb and PCV (Table 1). Thrombocyte count or platelet count was significantly (P<0.05) decreased in NMU control group-C rats when compared to group A, B, D and E rats .Whereas, *Z. jujube* treated treatment group-D and prevention group-E rats showed normal levels of platelet count (Table 1). Also, The NMU control group-C rats showed significant (P<0.05) increase in lymphocytes and neutrophils indicating lymphocytosis and neutrophilia, respectively, when compared to group B, D and E rats. *Z. jujube* treated treatment group-D and prevention group-E rats showed significant decrease in lymphocytes and neutrophils and exhibited normal WBC levels (Table 1).

3.3.4. Serum biochemical parameters

The effects of *Z. jujube* treatment in terms of serum AST, ALT, ALP, LDH, total protein, albumin and calcium of rats in different groups are presented in Table 2. Group-C rats showed significant (P<0.05) decrease in albumin and total protein levels indicating hypoproteinemia in comparison to groups-A, B, D and E rats. Whereas *Z. jujube* treated groups-D and E rats showed normal levels of albumin and total protein. Also our results showed significant (P<0.05) decrease of AST and ALT enzymes in the serum of group-C rats as compared with other groups. In all groups it was found that the calcium in the serum dose not differ significantly. NMU treated groups C rats showed significant increase in the serum LDH and ALP levels as compared to other groups.

4. Discussion

Cancer is a major health problem in both developed and developing countries. Because of the high death rate associated with cancer and because of serious the side effects of chemotherapy and radiation therapy, many cancer patients seek alternative complementary methods of treatment. Natural products have played an important role in treating and preventing human diseases such as cancer, and have been derived from various source materials, especially terrestrial plants. The importance of plants with few side effects for use as antitumor agents in modern medicine has been

Table 1
Effect of aqueous extract of *Z. jujube* fruit on hematological values in different experimental groups.

CBC parameters	Group A	Group B	Group C	Group D	Group E
RBC ($\times 10^6/\mu$ L)	7.36± 0.20	7.25±0.21	5.37±0.18[a]	7.32± 0.21	7.61± 0.19
WBC($\times 10^3/\mu$ L)	6.65± 0.52	6.82±0.35	14.73±0.59[a]	10.56± 0.41[b]	7.84± 0.46[c]
HGB (g/dL)	14.12± 0.11	14.58±0.42	11.14±0.19[a]	13.29± 0.25	14.6± 0.15[c]
PCV %	45.32± 0.44	45.10±0.35	39.30±0.56[a]	42.07± 0.77	44.36± 0.44
LYM %	84.35± 0.55	83.40±0.51	149.90±0.70[a]	115.60± 0.68[b]	96.11±0.72[c]
NEU %	11.03± 0.36	11.08±0.25	22.39±0.45[a]	16.31± 0.24[b]	13.30± 0.33[c]
PLT($\times 10^3/\mu$ L)	710.43± 3.90	711.20±3.30	605.37± 2.80[a]	650.45± 4.30[b]	710.22± 5.10

Values are presented as mean ± SD; [a]P<0.05; in comparison with control group (A); [b]P<0.05; in comparison with NMU-treatment group (C); [c]P<0.05; in comparison with *Z. jujube* control (B); Group A: vehicle control, group B: *Z. jujube* control, Group C: NMU control, Group D: *Z. jujube* treatment and Group E: *Z. jujube* prevention

Table 2
Effect of aqueous extract of *Z. jujube* fruit on biochemical parameters in different experimental groups.

Biochemical parameters	Group A	Group B	Group C	Group D	Group E
LDH (U/L)	320.00±1.80	305.00±2.20	1150.00±3.80[a]	730.00±2.80[b]	540.00±3.35[c]
ALP (U/L)	127.00±0.20	130.00±0.30	650.00±0.50[a]	320.00±0.20[b]	210.00±0.30[c]
AST (U/L)	140.00±0.85	141.00±1.00	128.00±1.10[a]	135.00±1.50	137.00±1.20
ALT (U/L)	38.00±0.15	38.00±0.22	34.00±0.25[a]	36.00±0.40[b]	37.00±0.30
Albumin (g/dL)	4.30±0.09	4.60±0.15	2.80±0.11[a]	3.90±0.18[b]	4.00±0.18[c]
Total Protein (g/dL)	7.10±0.12	7.10±0.11	5.10±0.20[a]	6.90±0.15	6.80±0.19[c]

Values are presented as mean ± SD; [a]P<0.05 in comparison with control group (A); [b]P<0.05 in comparison with NMU-treatment group (C); [c]P<0.05 in comparison with *Z. jujube* control (B); Group A: vehicle control, group B: *Z. jujube* control, Group C: NMU control, Group D: *Z. jujube* treatment and Group E: *Z. jujube* prevention.

discussed in recent reviews and reports[20–23]. Hence, finding a new natural source with anticancer activities would aid in finding new tools for cancer therapy[24–28]. In the present research, the effect of *Z. jujube* on breast cancer in both in vivo and in vitro experiments is investigated.

Z. jujube extraction demonstrated a significant inhibitory effect on the various cancer cells' proliferation[10–15]. Huang et al. reported that this fruit has shown anticancer activity on human liver cancer cells that are highly resistant to chemotherapy drugs[15]. Vahedi et al. showed that *Z. jujube* induced morphological changes, including cell shrinkage and detachment in the HeLa and Hep-2 cells[12]. Plastina and colleagues indicated the cytotoxic effects of *Z. jujube* fruit extracts on breast cancer cells by MTT assay[13]. According to in vitro data, we suggest that aqueous extract of *Z. jujube* fruit caused some cell morphological variation, such as changes in cell adhesion to the surface of the plate, shrinkage, condensation and deformation of the cell wall. Cells were exposed to increasing doses of *Z. jujube* extracts for 24, 48 and 72 hours, and cell viability was determined by MTT assay. As shown in Figure 1, cell viability in the breast cancer cell line was markedly decreased after exposure to aqueous *Z. jujube* extract in a dose- and time-dependent manner.

We also examined the induction of breast cancer in rats by N-Methyl-N-Nitrosourea (NMU) and the therapeutic effect of *Z. jujube* on that cancer for the first time in the world. NMU is a highly specific mammary gland carcinogen and induced mammary tumours model has been widely used to evaluate chemo-preventive and therapeutic agents for breast cancer in human[29–31]. In our study, the body weights of the animals differ throughout the experiment. Overall, weight loss is one of the most frequent symptoms reported in cancer and it is often seen as one of the late signs of tumour progression. According to the obtained data, there was only a significant difference between the average bodyweight of the control rats (235 g) and the group treated with 60 mg/kg of NMU (160 g). At the end of the experiment and after euthanizing the animals, some factors including total antioxidant capacity, biochemical parameters (albumin, total protein, alanine aminotransferases (ALT/SGPT), aspartate aminotransferases (AST/SGOT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and calcium) and haematology were determined in their serum.

It is well known that free radical production and subsequent oxidative stress play an important role in tumour initiation, promotion and progression[32]. The antioxidant agents are believed to protect against cancer by scavenging reactive radical species, resulting in a reduced level of radical-mediated DNA damage. According to the data shown in Figure 3, the control groups (A and B) have the highest values for plasma antioxidant capacity (FRAP) among the groups under study. The decreased serum antioxidant capacity due to cancer induction was increased after *Z. jujube* treatment. In fact, the anticancer properties of this plant may consist in its secondary metabolites, for instance flavonoids that have antioxidant properties[33]. More than 20 types of flavonoids have been isolated from the jujube fruit [34].

The haematological results showed significantly decreased in RBC, Hb and PCV levels in the NMU control group C rats, indicating a tendency to develop erythropaenia or anaemia. The group C rats also showed significantly increases in lymphocytes, indicating lymphocytosis with neutrophilia. This indicates an inflammatory response in animals with small mammary tumours. These findings were in agreement with previous studies which illustrated anaemia, neutrophilia and leukaemia in NMU-injected rats[35, 36]. The *Z. jujube* treatment group D and prevention group E showed normal levels of RBC, Hb, PCV, lymphocytes and neutrophils. Thus daily aqueous extract of Z. jujube (as a medicinal herb) at the dose of 400 mg/kg reduced anaemia, neutrophilia and early-stage leukaemia in rats.

Non-significant differences in the concentration of calcium and iron in sera samples before and after treatment with both NMU and *Z. jujube* are observed (data not shown). This means that calcium assay is not a proper test for early detection and follow-up of breast cancer in the treatment studies. Since none of the rats in our study were received in the last stage of breast cancer, the changes in serum calcium were not significant. In the present study, NMU-induced cancer is clearly evidenced by the marked elevation in serum ALP and LDH and the decreased levels of total protein and albumin in serum. These biochemical marker enzymes are indicators of tumour response and neoplastic process[37]. ALP is used as a general tumour marker during diagnosis in the early detection of cancer. Also, cancer induction causes cell injury, which was monitored by measuring the LDH released in the blood[38]. However, the increased serum LDH and ALP due to tumour promotion decreased significantly after *Z. jujube* treatment. These results firmly establish the role of *Z. jujube* in decreasing the severity of cancerous alteration in rats. Additionally, the low level of albumin in the serum can increase the pool of free radicals and may cause oxidant stress after cancer incidence[39]. Low serum albumin and total protein have also been shown to be independent indicators for prognosis in various cancers, like breast[40]. As seen in Table 2, the serum total protein, the albumin and the soluble hepatic enzymes like AST and ALT were increased by *Z. jujube* treatment.

In conclusion, both in vivo and in vitro results indicated that *Z.*

jujube had a cytotoxic effect on cancerous rats and cancer (MDA-MB-468) cells. Due to the fact that tumour progression is closely related to inflammation and oxidative stress, compounds that have antioxidant activity, such as *Z. jujube*, can be anti-carcinogens. Among different biochemical parameters, serum LDH and ALP levels, total protein and albumin were significantly changed by *Z. jujube* treatment. This indicates that the cellular destruction attributable to the tumour progression was controlled by this plant. Thus, our study illustrates the cancer chemoprevention and treatment properties of *Z. jujube* at a nontoxic concentration.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

[1] Krishnan P, Yan KJ, Windler D, Tubbs J, Grand R, Li BD, et al. Citrusauraptene suppresses cyclin D1 and significantly delays N-methyl nitrosourea induced mammary carcinogenesis in female Sprague-Dawley rats. *BMC Cancer* 2009; **9**(259): 1-12.

[2] Weiss RH, Marshall D, Howard L, Corbacho AM, Cheung AT, Sawai ET. Suppression of breast cancer growth and angiogenesis by an antisense oligodeoxynucleotide to p21 (Waf1/Cip1). *Cancer Lett* 2003; **189**(1): 39-48.

[3] Duss S, André S, Nicoulaz AL, Fiche M, Bonnefoi H, Brisken C, et al. An oestrogen-dependent model of breast cancer created by transformation of normal human mammary epithelial cells. *Breast Cancer Res* 2007; **9**(3): 1-15.

[4] De Vargas, Barbosa NB, Nogueira CW, Guecheva TN, Bellinaso Mde L, Rocha JB. Diphenyldiselenide supplementation delays the development of N-nitroso-N-methylurea-induced mammary tumors. *Arch Toxicol* 2008; **82**(9): 655-663.

[5] Mans DR, da Rocha AB, Schwartsmann G. Anti-cancer drug discovery and development in Brazil: targeted plant collection as a rational strategy to acquire candidate anticancer compounds. *Oncologist* 2000; **5**(3):185–198

[6] Omidbaigi R, Daghighi S. *Effects of sucker age and transplanting time on the propagation of jujube tree*. Paper presented at the III WOCMAP congress on medicinal and aromatic plants, Thailand; 2005.

[7] Jagadeesan AJ, Langeswaran K, Gowthamkumar S, Revathy R, Balasubramanian MP. Chempreventive potential of diosgenin on modulating glycoproteins, tca cycle enzymes, carbohydrate metabolizing enzymes and biotransormation enzymes against n-methyl-n-nitrosourea induced mammary carcinogenesis. *Int J Pharm Pharm Sci* 2013; **5**(4): 575-582

[8] Bown D. *Encyclopaedia of herbs and their uses*. London: Dorling Kindersley;1995.

[9] Him-Che Y. *Handbook of Chinese herbs and formulas*. Beijing: Institute of Chinese Medicine;1985, p. S219-S22

[10]Lee SM, Min BS, Lee CG. Cytotoxic triterpenoids from the fruits of *Zizyphus jujuba*. *Planta Med* 2003; **69**(11):1051-1054

[11]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.

[12]Vahedi F, Fathi Najafi M, Bozari K. Evaluation of inhibitory effect and apoptosis induction of *Zyzyphus jujube* on tumor cell lines, an *in vitro* preliminary study. *Cytotechnology* 2008; **56:** 105-111

[13]Plastina P, Bonofiglio D, Vizza D, Fazio A, Rovitob D, Giordano C, et al. Identification of bioactive constituents of *Ziziphus jujube* fruit extracts exerting antiproliferative and apoptotic effects in human breast cancer cells. *J Ethnopharmacol* 2012; **140:** 325-332.

[14]Chan ASL, Yip ECH, Yung LY, Pang HH, Luk SCW, Pang SF, et al. Immuno-regulatory effects of CKBM on the activities of mitogen-activated protein kinases and the release of cytokines in THP-1 monocytic cells. *Biol & Pharm Bull* 2005; **28:** 1645-1650

[15]Huang XD, Kojima-Yuasa A, Norikura T, Kennedy DO, Hasuma T, Matsui-Yuasa I. Mechanism of the anti-cancer activity of *Zizyphus jujuba* in HepG2 cells. *Am J Chin Med* 2007; **35:** 517-532.

[16]Huang XD, Kojima-Yuasa A, Xu SH, Norikura T, Kennedy DO, Hasuma T, et al. Green tea extract enhances the selective cytotoxic activity of Ziziphus jujuba extracts in HepG2 cells. *Am J Chin Med* 2008; **36:** 729-744.

[17]Huang XD, Kojima-Yuasa A, Xu SH, Kennedy DO, Hasuma T, Matsui-Yuasa I. Combination of *Ziziphus jujuba* and green tea extracts exerts excellent cytotoxic activity in HepG2 cells via reducing the expression of APRIL. *Am J Chin Med* 2009; **37:** 169-179.

[18]Zhou XM, Wong BC, Fan XM, Zhang HB, Lin MC, Kung HF, et al. Non-steroidal anti-inflammatory drugs induce apoptosis in gastric cancer cells through up-regulation of bax and bak. *Carcinogenesis* 2001; **22:** 1393-1397.

[19]Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 1996; 239(1): 70-76.

[20]Newman DJ, Cragg G. Snader KM. Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 2003; **66:** 1022-1037.

[21]Koehn FE, Carter GT. The evolving role of natural products in drug discovery. *Nat Rev Drug Discov* 2005; **4:** 206-220.

[22]Paterson I, Anderson EA. Chemistry. The renaissance of natural products as drug candidates. *Science* 2005; **310: 4**51-453.

[23]Bathaie SZ, Mousavi SZ . New applications and mechanisms of action of saffron and its important ingredients. *Crit Rev Food Sci Nutr* 2010; **50**(8): 761-786.

[24]Shahneh FZ, ValiyarI S, Azadmehr A, Hajiaghaee R, Bandehagh A, Baradaran B. *In vitro* cytotoxic and apoptotic activity of four Persian medicine plants on human leukemia and lymphoma cells. *Asian Pac J Trop Dis* 2014; **4:** s415-s420.

[25]Parvathaneni M, Rao Batt G, Gray A, Gummalla P. Investigation of anticancer potential of hypophyllanthin and phyllanthin against breast cancer by *in vitro* and *in vivo* methods *Asian Pac J Trop Dis* 2014; **4:** s71-s76.

[26]Bathaie SZ, Hoshyar R, Miri HR, Sadeghizadeh M. Anticancer effects of crocetin in both human adenocarcinoma gastric cancer cells and rat model of gastric cancer. *Biochem Cell Biol* 2013; **91:** 1-7.

[27]Hoshyar R, Bathaie SZ, Sadeghizadeh M. Crocin triggers the apoptosis through increasing the Bax/Bcl-2 ratio and caspase activation in human gastric adenocarcinoma, AGS, cells. *DNA and Cell Biol* 2012; **32**(2): 50-57.

[28]Waheed A, Bibi Y, Nisa S, Chaudhary F, Sahreen S, Zia M. Inhibition of human breast and colorectal cancer cells by *Viburnum foetens* L. extracts *in vitro*. *Asian Pac J Trop Dis* 2013; **3:** 32-36.

[29]Mehta RG. Experimental basis for the prevention of breast cancer. *Eur J Cancer* 2000; **36:** 1275-1282

[30]Roomi MW, Roomi NW, Ivanov V, Kalinovsky T, Niedzwieck A Rath. Modulation of NMU induced mammary tumors in Sprague-Dawley rats by comination of lysine proline arginine ascordic acid and green tea extract. *Breast Cancer Res* 2005; **7:** R291-R295.

[31]Ashrafi M, Bathaie SZ, Abroun S. High expression of Cyclin D1 and p21 in N-Nitroso-N-Methylurea-Induced breast cancer in Wistar albino female rats. *Cell J* (Yakhteh) 2012; **14:** 193-20

[32]Suzuki YS, Forman JJ, Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 1997; **22:** 269-285.

[33]Middleton E Jr. Effect of plant flavonoids on immune and inflammatory cell function. *Adv Exp Med Biol* 1998; **439:** 175-82.

[34]Cheng G, Bai Y, Zhao Y, Tao J, Liu Y, Tu G, et al. Flavonoids from *Ziziphus jujube* Mill var. spinosa. *Tetrahedron* 2000; **56:** 8915-8920

[35]Perse M, Cerar A, Injac R, Strukelj B. NMU induced breast cancer in rat, the histopathology of the resulting tumors and its drawbacks as a model. *Pathol Oncol Res* 2009; **15:** 115-121.

[36]Chang YC, Hsu JD, Lin WL, Lee YJ, Wang CJ. High incidence of acute promyelocytic leukemia specificialy induced by NMU in Sprague-dawley rats. *Arch Toxicol* 2012; **86:** 315-327.

[37]Thirunavukkarasu C, Sakthisekaran D. Sodium selenite modulates tumour marker indices in N-nitrosodiethylamine initiated and phenobarbital-promoted rat liver carcinogenesis. *Cell Biochem Funct* 2003; **21:** 147-153.

[38]Mei Y, Wei D, Liu J. Modulation effect of tea polyphenol toward N-methyl-N'-nitro-N-nitrosoguanidine-induced precancerous gastric lesion in rats. *J Nutr Biochem* 2005; **16:** 172-177.

[39]Abuja PM, Albertini R. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clinica Chimica Acta* 2001; **306** (1-2): 1-17.

[40]Lis CG, Grutsch JF, Vashi PG, Lammersfeld CA. Is serum albumin an independent predictor of survival in patients with breast cancer? *JPEN J Parenter Enteral Nutr* 2003; **27:** 10-15.