RESEARCH ARTICLE

Anti-mutagenic Activity of *Salvia merjamie* Extract Against Gemcitabine

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Abstract

Gemcitabine is an anti-cancer drug with clinically uses in the treatment of various neoplasms, including breast, ovarian, non-small cell lung, pancreaticand cervical cancers, T-cell malignancies, germ cell tumours, and hepatocellular carcinomas. However, it has also been reported to have many adverse effects. Naturally occurring anti-mutagenic effects, especially those of plant origin, have recently become a subject of intensive research. The present study was therefore designed to investigate the anti-mutagenic effects of *Salvia merjamie* (Family: Lamiaceae) plant extracts against the mutagenic effects of gemcitabine. The anti-mutagenic properties of *Salvia merjamie* were tested in Inbred SWR/J male and female mice bone marrow cells. The mice were treated in four groups; a control group treated with 30 mg/kg body weight gemcitabine and three treatment groups, each with 30 mg/kg body weight gemcitabine together with, respectively, 50, 100 and 150 mg/kg body weight *Salvia merjamie* extract. Chromosomal aberration and mitotic index assays were performed with the results demonstrating that *Salvia merjamie* extract protects bone marrow cells in mice against gemcitabine induced mutagenicity. This information can be used for the development of a potential therapeutic anti-mutagenic agents.

Keywords: Gemcitabine - *Salvia merjamie* - chromosomal aberration - mitotic index

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Introduction

Gemcitabine, is a nucleoside analogue antineoplastic used in chemotherapy. It is classified as an anti-metabolite in the subclass of pyrimidine analogues and metabolizes intracellularly to activate the diphosphate and triphosphate nucleosides (Yao et al., 2010; Suprasert et al., 2012). Gemcitabine inhibits thymidylate synthetase, leading to inhibition of DNA synthesis and cell death (Fowler et al., 2008). This anti-cancer drug is used clinically to treat various cancers, including breast cancer (Morandi, 2006), ovarian cancer (Lorusso et al., 2006), non-small cell lung cancer (Crino et al., 1999), pancreatic cancer (Burris et al., 1997), T-cell malignancies (Sallah, 2001), germ cell tumour (Einhorn et al., 1999), hepatocellular carcinomas (Kubicka et al., 2001), advanced squamous cell carcinoma of the head and neck (Catimel et al., 1994), cervical cancer (Mutch and Bloss, 2003), refractory transitional cell carcinoma of the bladder (Dalbagni et al., 2006) and peritoneal mesothelioma (Fracasso et. al., 1999). Gemcitabine has also been reported to have adverse effects including, haematological toxicity (Crombag et al., 2014) and pulmonary toxicity (Barlesi et al., 2004; Chi et al, 2012). Furthermore, gemcitabine has been reported to have mutagenic activity in male albino mice *in vivo* (Mohammed et al., 2009) and in human lymphocytes *in vitro* (Aydemir et al., 2005). Mutation is an essential factor in carcinogenesis and the occurrence of cancers may be reduced by decreasing the rate of the mutations. The best approach to decrease the rate of mutation in humans is to avoid exposure to mutagens and carcinogens (Kim et al., 2000). Plant derived natural products have received considerable attention since ancient times due to their potent antioxidant activity and diverse pharmacological and anticancer properties (Ong et al., 1986; Owen et al., 2004; Omar, 2010; Patel et al., 2010; Karmakar et al., 2010; Al-Oqail et al., 2013; Al-Sheddi et al., 2014; Farshori et al., 2013 and 2014) and there is, therefore, a pressing need to identify and investigate plant derived compounds with potential anti-carcinogenic and anti-mutagenic properties. Various studies have demonstrated that plant extracts have anti-mutagenic activity (Taneja et al., 2003; Meena et al., 2006; Agrawal and Pandey, 2009; Kumar et al., 2010) and the present study was designed to investigate the mutagenic effects of *Salvia merjamie* (Family: Lamiaceae)plant extracts against the mutagenic effects of gemcitabine.

Materials and Methods

Experimental animals

Inbred SWR/J male and female mice (10-12 weeks old and weight range 29.2-31.8 gm) were used in the present study. Animals were obtained from the Experimental

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Animal Care Center, King Saud University, Riyadh and were maintained in an environmentally controlled room at a temperature of 22±1ºC, a relative humidity of 45±5 on a 10/14h light/dark cycle with standard food pellets and drinking water *ad libitum*. All experiments on animals were carried out according to the Guidelines of the Animal Care and Use Committee, King Saud University, Kingdom of Saudi Arabia.

Gemcitabine preparation

One gram of gemcitabine powder (BDH chemical) was dissolved in 100 ml of sterile normal saline, and then 30 mg/kg body weight was applied.

Plant material

The flowering twig of *Salvia merjamie* growing wildly in nature was collected along with voucher specimens from Medina regions of Saudi Arabia. The plants were identified through consultation of the flora of Saudi Arabia (Chaudhary, 2001), and a specimen was submitted to the Herbarium of King Saud University in Riyadh, Saudi Arabia. The collected plant materials were rinsed thoroughly with tap water to remove extraneous contaminants and were then cut into small pieces, oven-dried at 50°C until the dry weight stabilized, and ground into a powder with an electric grinder. A crude extract was prepared by macerating the powdered plant materials (1000 g) in 95% ethanol at room temperature for 1 week. Extracts were filtered and concentrated using a rotary evaporator at low temperature and pressure. The crude extracts were prepared in normal saline and were applied orally.

Experimental design

Group I): Gemcitabine (30 mg/kg body weight); *Group II)*: Gemcitabine (30 mg/kg body weight) + *Salvia merjamie* extract (50 mg/kg body weight); *Group III)*: Gemcitabine (30 mg/kg body weight) + *Salvia merjamie* extract (100 mg/kg body weight); *Group IV)*: Gemcitabine (30 mg/kg body weight) + *Salvia merjamie* extract (150 mg/kg body weight).

For each treatment group, mice were sacrificed after 24, 48 and 72h for analysis while anesthetized.

Chromosomal aberration test

Chromosome Preparations: Chromosomal preparations were performed following the methods of Preston et al. (1987) and Al-Hawary and Al-Saleh, 1989.

Slide preparations: A minimum of ten slides were prepared and distinctly identifiable metaphases were selected from each mouse. Each selected metaphase was examined using the 100xoil immersion objective of a Zeiss microscope in order to detect possible chromosomal aberrations. Prior to scoring the drug's effect on the chromosomes, the slides were covered and coded. The chromosomal aberrations scanned were: chromatid gaps (G), chromatid breaks (B), fragments (F), ring chromosomes (R), deletions (D), centromeric attenuation (CA), centric fusion (CF), pulverized chromosomes (PC), and End to End association (EE). According to the criterion of Matsuoka et al. (1979), a complete discontinuity narrower than the width of a chromatid was considered to be a gap. Photomicrographs of selected metaphases were taken under bright illumination usingthe 100xoil immersion objective and a 10xeyepiece.

Mitotic index

The mitotic index (MI) was determined using the protocol of Shubber and Juma (1999), scoring at least 1000 cells from each animal, and the MI was then calculated through the ratio of mitotic cells to interphase in 1000 cells.

Mitotic index (MI %)=Number of dividing cells / total No. of cells scored X 100

Statistical analysis

The results expressed as mean±SE were statistically analysed using a SAS computer program and a student-t test (Sokal and Rohlf, 1981).

Results

Effects of Salvia merjamie extracts on chromosomal aberrations in mice bone marrow cells induced by gemcitabine

The results of the frequencies of chromosomal aberrations induced by gemcitabine and the preventive effects of *Salvia merjamie* extract are summarized in Tables 1-3 and Figure 1. A statistically significant dose- and time-dependent effect of *Salvia merjamie* extract on chromosomal aberration was observed. As shown in Table 1, while gemcitabine increased the number of chromosomal aberrations, in comparison, the mice treated with *Salvia merjamie* at 100 and 150 mg/kg body weight for 24h exhibited a significantly decreased number of abnormal cells. The effect of *Salvia merjamie* extract was found to become more marked as the length of exposure increased. As shown in Table-2 and 3, however, a significant effect was observed even at the lowest dose, i.e. 50 mg/kg body weight of *Salvia merjamie* extract.

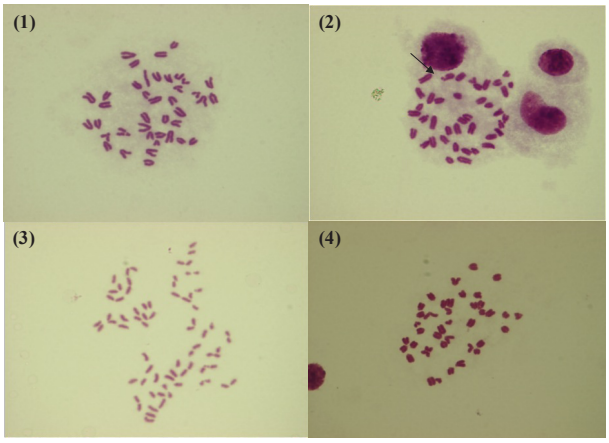

Figure 1. Representative Images of Mice Bone Marrow Cells Showing Metaphase Stages in *Salvia merjamie* and Gemcitabine-treated Mice after 24h. (1) Normal metaphase stage; **(2)** Metaphase breakage; **(3)** Centromeric attenuations in salvia and gemcitabine treated animals after 24h; **(4)** Centric Fusion In metaphase

Table 1. Antimutagenic Effects of *Salvia merjamie* Extract Against the Gemcitabine Altered Chromosomal Aberrations in SWR/J mice after 24h injection

Dose			Numerical chromosomal aberrations		No. and types of structural chromosomal aberrations									Total numerical chromosomal aberrations			
(mg/kg)	No. of animals used	No. of cells examined	No.of cells with one aberrations	No.of cells with more aberrations	Total		G	B	F	D	CA	CF	PC	EE	With G		Without G	
					No	%									No	%	N	%
G 30	6	600	57	11	68	11.3	9	13	11	6	10	7	8	4	68	11.3	59	9.8
G+V 50	6	600	60	6	66	11	6	10	6	10	9	9	8	8	66	11	60	10
G+V 100	6	600	39	3	42	7	4	17	5	9	0	6	0	1	42	7	38	63**
G+V 150	6	600	22	1	23	3.8	0	13	2	4	0	1	3	0	23	3.8	23	33**

**G=Gap; B=Breek; F=Fragment; D=Deletion; CA=Centromeric Attenuation; CF=Centric Fusion; PC=Pulverized Chromosomes; EE=End to End association*

Table 2. Antimutagenic Effects of *Salvia merjamie* Extract Against the Gemcitabine Altered Chromosomal Aberrations in SWR/J mice after 48h injection

Dose			Numerical chromosomal aberrations		No. and types of structural chromosomal aberrations									Total numerical chromosomal aberrations			
(mg/kg)	No. of animals used	No. of cells examined	No.of cells with one aberrations	No.of cells with more aberrations	Total		G	B	F	D	CA	CF	PC	EE	With G		Without G	
					No	%									No	%	N	%
G 30	6	600	50	4	54	9	4	11	9	6	7	9	4	4	54	9	50	8.33
G+V 50	6	600	35	1	36	6	5	6	3	5	3	4	6	4	36	6	31	5.17**
G+V 100	6	600	24	3	27	4.5	5	6	4	2	1	5	3	1	27	4.5	22	3.67**
G+V 150	6	600	22	2	24	4	5	5	4	3	2	4	0	1	24	4	19	3.17**

**G=Gap; B=Breek; F=Fragment; D=Deletion; CA=Centromeric Attenuation; CF=Centric Fusion; PC=Pulverized Chromosomes; EE=End to End association*

Table 3. Antimutagenic Effects of *Salvia merjamie* Extract Against the Gemcitabine Altered Chromosomal Aberrations in SWR/J mice after 72h injection

Dose			Numerical chromosomal aberrations		No. and types of structural chromosomal aberrations									Total numerical chromosomal aberrations			
(mg/kg)	No. of animals used	No. of cells examined	No.of cells with one aberrations	No.of cells with more aberrations	Total		G	B	F	D	CA	CF	PC	EE	With G		Without G	
					No	%									No	%	N	%
G 30	6	600	26	0	26	4.3	2	6	1	6	1	3	2	5	26	4.3	24	4
G+V 50	6	600	20	2	22	3.6	4	4	2	4	1	4	0	3	22	3.6	18	3
G+V 100	6	600	16	1	17	2.8	4	16	6	2	8	5	0	4	17	2.8	13	2.2**
G+V 150	6	600	19	0	19	3.2	2	12	2	1	5	4	0	1	19	3.2	17	2.8 **

**G=Gap; B=Breek; F=Fragment; D=Deletion; CA=Centromeric Attenuation; CF=Centric Fusion; PC=Pulverized Chromosomes; EE=End to End association*

Effects of Salvia merjamie extracts on changes in the mitotic index in mice bone marrow cells induced by gemcitabine

The effect of different concentrations of *Salvia merjamie* extract and gemcitabine on mice bone marrow mitotic index frequencies are shown in Tables 4-6. Compared to mice treated with gemcitabine alone, those treated with a combination of *Salvia merjamie* extract and gemcitabine showed a statistically significant increase in bone marrow mitotic indices. There was a significant ($p>0.01$) difference in the mitotic indices between all the studied groups. The mice treated with gemcitabine alone showed a mitotic index of 2.2% at 24h, whereas the mitotic index of mice treated with *Salvia merjamie* extract at100 and 150 mg/kg increased to 3.6% and 4.1%, respectively. Similarly, mice treated with100 and 150 mg/kg of *Salvia merjamie* extract had mitotic indices of3.5% and 3.7%, respectively, at 48h and 3.6% and 3.8%, respectively, at 72h, whereas those treated with gemcitabine alone had mitotic indices of 2.2% and 3.2% at 48h and 72h. There was no significant effect on the mitotic index of those mice treated 50 mg/kg of Salvia extract at any of the time intervals, however.

Table 4. Effects of *Salvia merjamie* Extract on Mitotic Index of Bone Marrow Cells of SWR/J Mice After 24h of Injection

Dose (mg/kg)	No. of animals used	No. of cells examined	No. of dividing cells	Mitotic index (%)
GEMCITABINE 30 mg/kg	6	6000	130	2.2
GEMCITABINE +Salvia 50 mg/kg	6	6000	133	2.2
GEMCITABINE +Salvia 100 mg/kg	6	6000	216	3.6**
GEMCITABINE +Salvia 150 mg/kg	6	6000	244	4.1**

**Differences are statistically significant from the control group at p<0.01

Table 5. Effects of *Salvia merjamie* Extract on Mitotic Index of Bone Marrow Cells of SWR/J Mice After 48h of Injection

Dose (mg/kg)	No. of animals used	No. of cells examined	No. of dividing cells	Mitotic index (%)
GEMCITABINE 30 mg/kg	6	6000	142	2.4
GEMCITABINE +Salvia 50 mg/kg	6	6000	137	2.3
GEMCITABINE +Salvia 100 mg/kg	6	6000	208	3.5**
GEMCITABINE +Salvia 150 mg/kg	6	6000	221	3.7**

**Differences are statistically significant from the control group at p<0.01

Table 6. Effects of *Salvia merjamie* Extract on Mitotic Index of Bone Marrow Cells of SWR/J Mice After 72h of Injection

Dose (mg/kg)	No. of animals used	No. of cells examined	No. of dividing cells	Mitotic index (%)
GEMCITABINE 30 mg/kg	6	6000	192	3.2
GEMCITABINE +Salvia 50 mg/kg	6	6000	190	3.2
GEMCITABINE +Salvia 100 mg/kg	6	6000	218	3.6 **
GEMCITABINE +Salvia 150 mg/kg	6	6000	229	3.8**

**Differences are statistically significant from the control group at p<0.01

Discussion

Salvia herbs belong to the Labiatae family of plants, which includes nearly 900 species spread throughout the world (Mozafarian, 1996). Plants that belong to this family are well known for their pharmacological and other bioactivities, and have often been used in traditional medicine (Xu, 1990). Hohmann et al. (1999) and Zupko et al. (2001) reported the antioxidant activities of many species of Salvia and their active constituents in enzyme-dependent and enzyme-independent systems. Phytochemical analyses of Salvia species show the presence of many compounds, mainly belonging to the phenolic acids, phenolic glycosides, flavonoids, anthocyanins, coumarins, polysaccharides, sterols, terpenoids and essential oils (Ghannadi et al., 1999; Lu and Foo, 2002). Several species of Salvia have been used to treat microbial infections, cancer, malaria, inflammation, loss of memory, as well as to disinfect homes after sickness (Kamatou et al., 2008). The present investigation aimed to assess the anti-mutagenic activity of *Salvia merjamie* extracts against the mutagenic effects of gemcitabine in bone marrow cells. Chromosomal aberrations and a decline in the mitotic index are the most sensitive indicators of bone marrow damage (Giri et al., 1988; Natarajan et al., 1993; Smalinskiene et al., 2005) and, therefore, the experiment was designed to observe whether the toxic effects induced by gemcitabine, as revealed by chromosomal aberrations and the mitotic index, were neutralized by the administration of *Salvia merjamie* extracts. Such anti-mutagenic and immunomodulatory activities of *Salvia merjamie* in respect to the mutagenicity induced by gemcitabine have not yet been evaluated.

As this study has shown, gemcitabine causes disturbed homeostasis and the induction of biological stress, which is manifested by a sharp decline in the mitotic index and an elevation of chromosomal aberrations. Our results are similar to those of other studies into gemcitabine. Salem et al. (2012), for example, have shown the cytotoxic effects of gemcitabine, while Aydemir and Bilaloglu (2003) and Aydemir et al. (2005) have used the structural chromosomal aberration assay and micronucleus test system and Fowler et al. (2009) have used DNA polymerization to show its genotoxic potential. In this study, however, experimental animals treated with a single dose of gemcitabine (30 mg/kg/day) but subsequently treated with *Salvia merjamie* for 24-72h showed a significant reduction in the mitotic index, indicating that *Salvia merjamie* is effective in reducing the mitotic index. The mice treated with gemcitabine and *Salvia merjamie* also showed a significant decrease in the number of chromosomal aberrations. The mitotic index of mice treated with *Salvia merjamie* extract in fact recovered to the point that it was equivalent to the mitotic index of the control group, and chromosomal damage was also significantly repaired by *Salvia merjamie* extract. It can be concluded, therefore, that *Salvia merjamie* extract protects mice bone marrow cells from gemcitabine induced mutation.

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References

Agrawal RC, Pandey S (2009). Evaluation of anticarcinogenic and antimutagenic potential of *Bauhinia variegata* extract in Swiss albino mice. *Asian Pac J Cancer Prev*, **10**, 913-16.

Al-Hawary, BA, Al-Saleh AA (1989). Cytogenetic effects of dacarbazine on mouse bone marrow cells *in vivo*. *Mut Res*, **223**, 259-66.

Al-Oqail MM, Farshori NN, Al-Sheddi ES, et al (2013). *In vitro* cytotoxic activity of seed oil of fenugreek against various cancer cell lines. *Asian Pac J Cancer Prev*, **14**, 1829-32.

Al-Sheddi ES, Farshori NN, Al-Oqail MM, et al (2014). Cytotoxicity of *Nigella sativa* seed oil and extract against human lung cancer cell line. *Asian Pac J Cancer Prev*, **15**, 983-7.

Aydemir N, Celikler S, Bilaloglu R (2005). *In vitro* genotoxic effects of the anticancer drug gemcitabine in human lymphocytes. *Mutat Res*, **582**, 35-41.

Aydemir N, Bilaloglu R (2003). Genotoxicity of two anticancer drugs, gemcitabine and topotecan, in mouse bone marrow *in vivo*. *Mutat Res*, **537**, 43-51.

Barlesi F, Villani P, Doddoli C, et al (2004). Gemcitabine-induced severe pulmonary toxicity. *Fund Clin Pharmacol*, **18**, 85-91.

Burris HA III, Moore MJ, Andersen J, et al (1997). Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol*, **15**, 2403-13.

Catimel G, Vermorken JB, Clavel M (1994). A phase II study of Gemcitabine (LY 188011) in patients with advanced squamous cell carcinoma of the head and neck. EORTC early clinical trials group. *Ann Oncol*, **5**, 543-47.

Chaudhary S (2001). Flora of the Kingdom of Saudi Arabia (Vascular Plants)," National Agriculture and Water Research Center, National Herbarium, Ministry of Agriculture and Water, Riyadh.

Chi DC, Brogan F, Turenne I, et al (2012). Gemcitabine-induced pulmonary toxicity. *Anticancer Res*, **32**, 4147-9.

Crino L, Scagliotti GV, Ricci S, et al (1999). Gemcitabine and cisplatin versus mitomycin, ifosfamide, and cisplatin in advanced non-small-cell lung cancer: A randomized phase III study of the Italian lung cancer project. *J Clin Oncol*, **17**, 3522-30.

Crombag MR, de Vries Schultink AH, Schellens JH, et al (2014). Incidence of hematologic toxicity in older adults treated with gemcitabine or a gemcitabine-containing regimen in routine clinical practice: a multicenter retrospective cohort study. *Drugs Aging*, **31**, 737-47.

Dalbagni G, Russo P, Bochner B, et al (2006). Phase II trial of intravesical gemcitabine in bacille Calmette-Guerin-refractory transitional cell carcinoma of the bladder. *J Clin Oncol*, **24**, 2729-34.

Einhorn LH, Stender MJ, Williams, SD (1999). Phase II trial of gemcitabine in refractory germ cell tumors. *J Clin Oncol*, **17**, 509-11.

Farshori NN, Al-Sheddi ES, Al-Oqail MM, et al (2013). Anticancer activity of *Petroselinum sativum* seed extracts on MCF-7 human breast cancer cells. *Pac J Cancer Prev*, **14**, 5719-23.

Farshori NN, Al-Sheddi ES, Al-Oqail MM, et al (2014). Cytotoxicity assessments of *Portulaca oleracea* and *Petroselinum sativum* seed extracts on human hepatocellular carcinoma cells (HepG2). *Asian Pac J Cancer Prev*, **15**, 6633-38.

Fowler JD, Brown JA, Johnson KA, et al (2008). Kinetic investigation of the inhibitory effect of gemcitabine on DNA polymerization catalyzed by human mitochondrial DNA polymerase. *J Biol Chem*, **283**, 15339-48.

Fracasso PM, Tan BR Jr, Grieff M, et al (1999). Membranoproliferative glomerulonephritis following gemcitabine and vinorelbine chemotherapy for peritoneal mesothelioma. *J Natl Cancer Inst*, **91**, 1779-80.

Ghannadi A, Samsam-Shariat SH, Moattar F (1999). Volatile constituents of the flower of Salvia hydrangea DC. *Ex Benth Daru*, **7**, 23-5.

Giri AK, Sharma A, Talukder G (1988). Relative efficancy of short term tests in detecting genotoxic effects of cadmium chloride in mice *in vivo*. *Mutat Res*, **206**, 285-95.

Hohmann J, Zupko I, Redei D, et al (1999). Protective effects of aerial parts of Salvia officinalis, Melissa officinalis, and Lavandula angustifolia and their constituents against enzyme-dependent and enzyme independent lipid peroxidation. *Planta Med*, **65**, 576-78.

Kamatou GPP, Makunga NP, Ramogola WPN, et al (2008). South African Salvia species: a review of biological activities and phytochemistry. *J Ethnopharmacol*, **119**, 664-72.

Karmakar SR, Biswas SJ, Khuda-Bukhsh AR (2010). Anti-carcinogenic potentials of a plant extract (*Hydrastis canadensis*): I. Evidence from *in vivo* studies in mice (Mus musculus). *Asian Pac J Cancer Prev*, **11**, 545-51.

Kim SY, Shon YH, Lee JS, et al (2000). Antimutagenic activity of soybeans fermented with basidiomycetes in Ames/Salmonella test. *Biotech Lett*, **22**, 1197-202.

Kubicka S, Rudolph KL, Tietze MK, et al (2001). Phase II study of systemic gemcitabine chemotherapy for advanced unresectable hepatobiliary carcinomas. *Hepatogastroenterology*, **48**, 783-89.

Kumar M, Meena P, Verma S, et al (2010). Anti-tumour, Anti-mutagenic and Chemomodulatory Potential of Chlorophytum borivilianum. *Asian Pac J Cancer Prev*, **11**, 327-34

Lorusso D, Di Stefano A, Fanfani F, et al (2006). Role of gemcitabine in ovarian cancer treatment. *Ann Oncol*, **17**, 188-94.

Lu Y, Foo LY (2002). Polyphenolics of Salvia-a review. *Phytochemistry*, **59**, 114-40.

Matsuoka A, Hayashi M, Ishidate M (1979). Chromosomal aberration test on 29 chemicals combined with 5q mix *in vitro*. Mutat Res, **60**, 277-90.

Meena PD, Kaushik P, Shukla S, et al (2006).Anticancer and antimutagenic properties of *Acacia nilotica* (Linn.) on 7,12-dimethylbenz(a)anthracene-induced skin papillomagenesis in swiss albino mice. *Asian Pac J Cancer Prev*, **7**, 627-32.

Mohammed BM, Karim KJ, Yaseen NY (2009). Antimutagenic effects of Thymus syriacus extract against the genotoxicity of gemcitabine in male albino mice. *The 2nd Kurdistan Conference on Biological Sciences J Duhok Univ*, **12**, 216-26.

Morandi P (2006). Biological agents and gemcitabine in the treatment of breast cancer. *Ann Oncol*, **17**, 177-80.

Mozafarian V (1996). A dictionary of Iranian Plant Names (Latin English Persian). Farhang Mosafer Publication, Tehran.

Mutch DG, Bloss JD (2003). Gemcitabine in cervical cancer. *Gynecol Oncol*, **90**, 8-15.

Natarajan, A, Duivenvoorden W, Meijers M, et al (1993). Induction of mitotic aneuploidy using Chinese hamster primary embryonic cells. test results of 10 chemicals. *Mutat Res*, **287**, 47-56.

Omar SH (2010). Oleuropein in olive and its pharmacological effects. *Sci Pharm*, **78**, 133-54.

Ong T, Wong W, Stwart JD (1986). Chlorophyllin a potent antimutagen against environmental and dietary complex mixture, *Nutr Res*, **173**, 111-5.

Owen RW, Haubner R, Wurtele G, et al., (2004). Olives and olive oil in cancer prevention. *Eur J Cancer Prev*, **13**, 319-26.

Patel VR, Patel PR, Kajal SS (2010). Antioxidant activity of some selected medicinal plants in western region of India. *Advan Biol Res*, **4**, 23-6.

Preston RJ, Dean BJ, Galloway S, et al (1987). Mammalian *in vivo* cytogenetic assays: analysis of chromosome aberrations in bone marrow cells. *Mutat Res*, **189**, 157-65.

Salem SD, Abou-Tarboush FM, Saeed NM, et al (2012). Involvement of p53 in gemcitabine mediated cytotoxicity

and radiosensitivity in breast cancer cell lines. *Gene*, **498**, 300-7.

Sallah S, Wan JY, Nguyen NP (2001). Treatment of refractory T-cell malignancies using gemcitabine. *Br J Haematol*, **113**, 185-87.

Shubber EK, Juma AS (1999). Cytogenetic effects of plants extract of Urtica dioca on mouse somatic cells. *Nucleus*, **42**, 182-87.

Smalinskiene A, Craileviciute R, Lesaukaite V, et al (2005). Effect of cadmium and Zinc ions on mitotic activity and protein synthesis in mouse liver. *Medicina (Kaunas)*, **41**, 506-11.

Sokal RR, Rohlf FJ (1981). Biometry: The Principles and Practice of Statistics in Biological Research". W.H. Freeman and Company, San Francisco, p. 859.

Suprasert P, Cheewakriangkrai C, Manopunya M (2012). Outcome of single agent generic gemcitabine in platinum resistant ovarian cancer, fallopian tube cancer and primary peritoneal adenocarcinoma. *Asian Pac J Cancer Prev*, **13**, 517-20.

Taneja P, Arora A, Shukla Y (2003). Antimutagenic effects of black tea in the *Salmonella typhimurium* reverse mutation assay. *Asian Pac J Cancer Prev*, **4**, 193-98.

Xu RS (1990). Biological and application of *Salvia miltiorrhiza* Bunge. *Science Press*, Beijing, pp. 23-177.

Yao CY, Huang XE, Tang JH, et al (2010). Clinical observations on safety of fixed dose rate gemcitabine chemotherapy by intravenous infusion. *Asian Pac J Cancer Prev*, **11**, 553-5.

Zupko I, Hohmann J, Redei D, et al (2001). Antioxidant activities of leaves of Salvia species in enzyme-dependent and enzyme independent systems of lipid peroxidation and their phenolic constituents. *Planta Med*, **67**, 366-8.