TÜBİTAK

Original Article

Turk J Med Sci
2011; 41 (1): 101-108
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0904-28

Urginea maritima (L.) Baker (Liliaceae) extract induces more cytotoxicity than standard chemotherapeutics in the A549 non-small cell lung cancer (NSCLC) cell line

Hakan BOZCUK[1], Mustafa ÖZDOĞAN[1], Oktay AYKURT[2], Fatih TOPÇUOĞLU[3], Hasan ÖZTÜRK[4], Deniz EKİNCİ[1], Asuman KARADENİZ[5], Aylin MUTLU[1], Durmuş BURGUCU[6]

Aim: *Urginea maritima* (Um) is a plant native to especially Turkey and the Mediterranean area. In this study, we investigated whether Um extract exerted cytotoxicity on cancer cells.

Materials and methods: We made various extracts of Um. These extracts in varying concentrations were added to an A549 NSCLC cell culture, alone or with gemcitabine and/or cisplatin. Growth inhibition was tested by the MTT assay and an Annexin V-FITC apoptosis detection kit

Results: The onion Um extract (1 µg/mL) was more cytotoxic than cisplatin (1 µg/mL), gemcitabine (1 nm/mL), and leaf Um extract (1 µg/mL) with $P < 0.001$, $P = 0.097$, and $P < 0.001$, respectively. The efficacy of Um extract was further improved by the addition of an antioxidant cocktail to an IC_{50} value of 0.02 µg/mL.

Conclusion: Um extract has been shown for the first time to be a strong candidate for drug development in solid tumors. Our studies continue to further define the specific antitumor compound(s) in this extract.

Key words: *Urginea maritima*, cytotoxicity, apoptosis, MTT, non-small cell lung cancer

Urginea maritima (L.) Baker (Liliaceae) ekstresinin A549 küçük hücreli dışı akciğer kanseri hücre hattında oluşturduğu, standart kemoterapötiklerden daha yüksek sitotoksisite

Amaç: *Urginea maritima* (Um) esas olarak Türkiye ve Akdeniz bölgesine özgü bir bitkidir. Biz bu çalışmada, Um ekstresinin kanser hücreleri üzerine sitotoksik etkisinin olup olmadığını araştırdık.

Yöntem ve gereç: Çeşitli Um ekstreleri yaptık. Bu ekstreler A549 Küçük Hücreli Akciğer Karsinomu (KHDAK) hücre kültürüne tek başına veya Gemsitabin ve/veya Sisplatin ile birlikte uygulandılar. Büyüme baskılanmasına MTT yöntemi ile ve Annexin V-FITC apopitoz kiti ile bakıldı.

Bulgular: (1 µg/mL) Um soğanı ekstresi, (1 µg/mL) Sisplatin, (1 nm/mL) Gemsitabin ve (1 µg/mL) Um yaprak ekstresinden daha sitotoksikdi ($P < 0,001$, $P = 0,097$, ve $P < 0,001$). Um ekstresinin etkinliği bir antioksidan kokteyl ilavesi ile 0,02 µg/mL düzeyine daha da arttırıldı.

Sonuç: Um ekstresinin solid tümörlerin tedavisi için geliştirilebilecek bir ilaç adayı olduğu literatürde ilk kez gösterildi. Çalışmalarımız bu ekstreden özgün antitümöral bileşiklerin tanımlanması yönünde devam etmektedir.

Anahtar sözcükler: *Urginea maritima*, sitotoksisite, apopitoz, MTT, küçük hücreli dışı akciğer karsinomu

Received: 30.04.2009 – Accepted: 08.03.2010

[1] Department of Medical Oncology, Faculty of Medicine, Akdeniz University, Antalya - TURKEY
[2] Faculty of Medicine, Akdeniz University, Antalya - TURKEY
[3] Department of Biology, Faculty of Science, Akdeniz University, Antalya - TURKEY
[4] Department of Biochemistry, Faculty of Medicine, Akdeniz University, Antalya - TURKEY
[5] Department of Biology, Faculty of Science and Arts, Mehmet Akif Ersoy University, Burdur - TURKEY
[6] Department of Hematology, Faculty of Medicine, Akdeniz University, Antalya - TURKEY

Correspondence: *Hakan BOZCUK, Akdeniz University, Dept. of Medical Oncology, Dumlupınar Boulevard, Antalya 07070 - TURKEY*
E-mail: hbozcuk@gmail.com

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide (1). Despite the advances in diagnosis and treatment of this disease, over the last 30 years the prognosis remains largely unchanged, and overall only 15% of patients presenting with NSCLC are cured (2).

Urginea maritima (Um), sea onion or white squill, is a native plant found in the Mediterranean area, North Africa, and India. This plant is known to exert diuretic and cardiotonic properties and has been used as a cough syrup (3). Additionally, Um is toxic and is known to cause human and animal poisoning (4,5) . Actually, compounds from this drug are also known to exhibit an insecticide effect (6). However, the antitumoral effect of this drug remains largely unexplored.

In the literature, we have not come across any data on the antitumoral effect of Um in solid tumors. A previous report showed that Um consisted of 33 chemical compounds, 10 of which were new (7). However, the specific functions and medical uses of these compounds are not totally clear at present. Therefore, since new and effective systemic treatments are eagerly awaited in the management in NSCLC, we aimed to investigate in this study the effect of Um in a NSCLC cell line, with the hope of identifying a new candidate for drug discovery in the management of NSCLC and other solid cancers.

Materials and methods

Extract preparation

For the purpose of extract preparation, only the Um plants growing far away from roads in the province of Antalya, Turkey, were utilized. Any environmental exposure to pesticides was excluded as only the plants from wild nature were used.

The multiple photographs and freshly picked forms of plants were presented to local and distant biologists and to the agriculture department before the experiments started. The methanol and water extracts were prepared as described in the literature, in 1 to 2 ratio (weight/weight; 15 g plant/60 g solvent), evaporated to dryness at 65 °C with a rotator evaporator and stored at -20 °C, with no exposure to light, until use (8). Separate extracts were prepared from the onion and leaf parts of the plant. Extracts were diluted with PBS (phosphate buffer saline) to obtain different concentrations of extracts in order to be used in the experiments.

Cell line and treatment of cells

The A549 NSCLC cell line was used for the cytotoxicity assays. Depending on the nature of the experiment, various treatments were applied to the cell line. At the first phase of the experiments, where the aim was to assess the impact of extract with regard to type of extract (derived from leaf versus onion), the following treatments were used:

1. Gemcitabine 1 nm/mL

2. Cisplatin 1 μg/mL

3. Extract (onion or leaf) 1 μg/mL (low dose)

4. Extract (onion or leaf) 100 μg/mL (high dose)

5. Gemcitabine 1 nm/mL + Extract (onion or leaf) 1 μg/mL

6. Gemcitabine 1 nm/mL + Extract (onion or leaf) 100 μg/mL

7. Cisplatin 1 μg/mL + Extract (onion or leaf) 1 μg/mL

8. Cisplatin 1 μg/mL + Extract (onion or leaf) 100 μg/mL

9. Control (no treatment, PBS alone)

At the second phase of the experiments, where the intention was to increase the activity of the extract, the following treatments were applied:

1. Aged onion extract (extract kept for a month, used as control), 1, 0.1, 0.01 μg/mL

2. N_2 modified onion extract, 1, 0.1, 0.01 μg/mL

3. Antioxidant cocktail modified onion extract, 1, 0.1, 0.01 μg/mL

The concentrations of gemcitabine and cisplatin as used in this study were chosen as it was previously shown that these concentrations were close to the IC50 value of these drugs in this cell line (9,10). The concentrations employed for the extract varied between 100 and 0.01 μg/mL.

MTT assay and cell culture

Growth inhibition of A549 cells by the treatments above was tested by the MTT (3-(4,5-dimethylthiazol-

2-yl)-2,5-diphenyl tetrasodium bromide) assay, as described in the literature, and as used in our previous work and in the literature (9,11,12). The MTT assay was used to indicate the level of cytotoxicity under some form of treatment with reference to control cells (without any treatment). The level of absorbance as assessed by spectrophotometry showed the amount of relative cytotoxicity. Briefly, cancer cells were seeded into 96-well microtiter plates at appropriate densities. After successful seeding, cells were exposed to various treatments as detailed above for 72 h. Tests were repeated 8 times. At the end of treatment, 20 µL of 5 mg/mL MTT (Sigma, St. Louis, MO, USA) was added to each well and the plates were incubated for 4 h at 37 °C. At the end, DMSO (150 µL) was added to each well and the optical absorbance at 570 nm was read on a plate reader. Cytotoxicity was defined as "100 × (1 – [(absorbance of treated cells)/(absorbance of control cells)]).

Assessment of apoptosis

According to the manufacturer's guidelines, an Annexin V-FITC apoptosis detection kit (Biovision Inc, Mountain View, CA, USA) was used to detect necrotic, early, and late apoptotic activity after 72 h of incubation. After various treatments, cells (1×10^6) were collected and resuspended in binding buffer, and Annexin V-FITC and propidium iodide were added to each sample and incubated in the dark for 5 min. Annexin V-FITC binding was determined by flow cytometry (Ex = 488 nm; Em = 530 nm) using an FITC signal detector (FL1) and propidium staining by the phycoerythrin emission signal detector (FL2).

Extract modification

In an attempt to augment the cytotoxicity by the onion extract, 2 modifications were made. The first one was the addition of N_2 gas to the extract with the aim of impeding the oxidative damage to compounds of the extract. The second modification consisted of the addition of an antioxidant cocktail made up of 3 compounds: mannitol (250 parts), sodium ascorbate (25 parts), and ascorbic acid (1 part), with reference to weight. These antioxidants have previously been used to stabilize similar extracts or solutions (13).

Statistical analysis

The magnitude of cytotoxicity with regard to different kinds of treatment, in relation to type of extract (onion or leaf), was compared in a general linear model, utilizing the Bonferroni procedure as a post hoc test. In addition, to investigate the effect of modifications on the efficacy of onion extract, the cytotoxicity induced was again compared in a multivariate model to test together the effects of type of modification, concentration of the extract, and their interaction term on the magnitude of cytotoxicity. Additionally, paired t testing was conducted to test the change in cytotoxicity in time. A P value < 0.05 was considered significant. SPSS 15.0.0 (SPSS Inc., Chicago, IL, USA, release 15.0.0) was used for the statistical analysis.

Results

The effect of treatment and type of extract on cytotoxicity

Various treatments yielded cytotoxicity results that were roughly between 40% and 80%. Extract from onion in low and high concentrations in particular yielded cytotoxicity levels above 80%. See Table 1 and Figure 1 for details.

When only type of extract was considered, extract derived from onion caused higher cytotoxicity over that from leaf with a difference of 8.5%, 78.7% versus 70.2% ($P < 0.001$). Likewise, when only type of treatment was considered (regardless of type of extract), extract in low dose was as effective as gemcitabine, and more effective than cisplatin, but less effective than extract in high dose, with P values of 1.000, <0.001, and <0.001, and cytotoxicity differences of 2.9%, 26.0%, and -7.6%, respectively. Data are not separately shown for univariate analyses.

When various treatments and type of extract (i.e. part of Um plant where extract is derived from) were considered in a multivariate model, type of treatment ($F = 107.0$, $P < 0.001$), type of extract ($F = 108.3$, $P < 0.001$), and type of treatment with respect to type extract ($F = 4.6$, $P < 0.001$) were all found to significantly affect the degree of cytotoxicity. Details are given in Table 2.

We also tested whether any type of extraction process (water or methanol extraction) can be better in terms of cytotoxicity. We found that methanol extraction was clearly more effective than water

Table 1. Descriptive statistics of cytotoxicity with respect to treatment and type of extract.

Treatment	Type of Extract	Mean (%)	Std. Deviation (%)	N (Test Repetitions)
Gemcitabine	Onion*	74.5	9.6	8
(1 nm/mL)	Leaf*	66.2	4.8	8
	Total	70.3	8.5	16
Cisplatin	Onion*	50.1	8.9	8
(1 μg/mL)	Leaf*	44.3	5.2	8
	Total	47.2	7.7	16
extract-low	onion	82.6	2.9	8
(1 μg/mL)	Leaf	63.9	2.6	8
	Total	73.2	10	16
extract-high	onion	84.2	2.7	8
(100 μg/mL)	Leaf	77.3	3.3	8
	Total	80.8	4.6	16
gem+extract-low	onion	82.4	2.8	8
(1 nm/mL + 1 μg/mL)	Leaf	74.8	2.7	8
	Total	78.6	4.8	16
gem+extract-high	onion	84.6	4.7	8
(1 nm/mL + 100 μg/mL)	Leaf	79.8	1.3	8
	Total	82.2	4.2	16
cisplatin+extract-low	onion	84.8	2.1	8
(1 μg/mL + 1 μg/mL)	Leaf	72.2	2.8	8
	Total	78.5	7	16
cisplatin+extract-high	onion	86.3	1.8	8
(1 μg/mL + 100 μg/mL)	Leaf	83.1	5.6	8
	Total	84.7	4.4	16

*: Cytotoxicity for gemcitabine only and cisplatin only treated cells represent pooled values derived from experiments where one specific type of extract is used.

extraction (details not given). Therefore, after the initial set of experiments, we always worked with methanol extracts. Therefore, our results as presented in this paper are derived from experiments where only methanol extraction was used.

Cytotoxicity with treatment when onion extract was separately used

After the demonstration that onion extract was more effective, a subgroup analysis was made for the tests where only onion extract with methanol extraction was used.

Type of treatment in this subgroup again influenced the amount of cytotoxicity ($F = 41.5$, $P < 0.001$). Cytotoxicity with the low dose extract was more than with cisplatin, and perhaps gemcitabine ($P < 0.001$, and 0.097, respectively). Refer to Table 3 for multiple comparisons with respect to treatment for this subgroup. Cytotoxicity by onion extract can again be viewed graphically in Figure 1.

Activity of extract in time

Activity of the low dose onion extract (1 μg/mL) was found to diminish slightly after a month

Modifications in extract composition and cytotoxicity

To counteract the decline in activity of the extract over time, 2 modifications were made. The first was

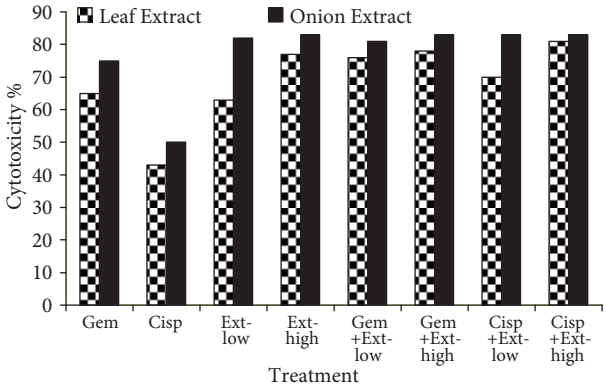

Figure 1. Cytotoxicity with respect to treatment and type of extract.
Cytotoxicity as measured by MTT by various treatments and with respect to the type of extract used. Gem: Gemcitabine (1 nm/mL), Cisp: Cisplatin (1 μg/mL), Ext-low: Extract (1 μg/mL), Ext-high: Extract (100 μg/mL).

the addition of N_2 gas, and the second was the inclusion of an antioxidant cocktail in the extract. For the aged only, aged with N_2, and aged with antioxidant cocktail type of extracts, the cytotoxicity values were 74.9%, 82.4%, and 76.8% at the 1 μg/mL level of extract; 29.6%, 58.3%, and 72.5% at the 0.1 μg/mL level of extract; and 12.7%, 25.3%, and 45.0% at the 0.01 μg/mL level of extract. Therefore, the activity was particularly enhanced with the addition of the antioxidant cocktail. Refer to Figure 2 for a diagrammatic representation of cytotoxicity caused by various concentrations of different modifications. Specifically, IC_{50} values for the aged only, aged with N2, and the aged with antioxidant cocktail extracts were around 0.22, 0.05, and 0.02 μg/mL, respectively.

The multivariate model confirms that type of modification, as well as the concentration, and the type of modification with respect to concentration, significantly determined the level of cytotoxicity (all with $P < 0.001$). See Table 4 for details.

Mechanism of cytotoxicity

Flow cytometric analysis using Annexin V revealed that the major mechanism of cytotoxicity was via the augmentation of apoptosis. Um leaf extract in low (1 μg/mL) and high (100 μg/mL) concentrations demonstrated apoptosis in 80% and 87.8% of cells, whereas Um onion extract in low (1 μg/mL) and high (100 μg/mL) concentrations caused apoptosis in 82.6% and 93.4%.

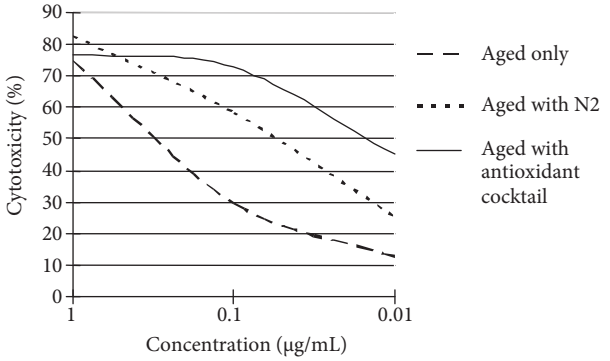

Figure 2. Cytotoxicity with extract modifications.
Assessment by MTT of the effect of different concentrations of extract with various extract modifications.

Table 2. The effect on the cytotoxicity of treatment and type of extract.

.

Dependent variable: cytotoxicity

Source	Type III sum of squares	df	Mean Square	F	P value
Corrected Model	18993.4(a)	15	1266.2	59.3	<0.001
Intercept	709106	1	709106	33232.8	<0.001
Treatment	15988.1	7	2284	107	<0.001
Type of extract	2311.7	1	2311.7	108.3	<0.001
Treatment × Type of extract	693.7	7	99.1	4.6	<0.001

(a) R Squared = 0.89 (Adjusted R Squared = 0.87)

Table 3. Comparisons of cytotoxicity by each treatment when only onion extract is used.

Multiple Comparisons

Dependent Variable Cytotoxicity (%)
Bonferroni

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
gemcitabine	cisplatin	24.3804*	2.6569	.000	15.6643	33.0964
	extract-low	-8.1102	2.6569	.097	-16.8262	0.6059
	extract-high	-9.7465*	2.6569	.015	-18.4626	-1.0305
	gem+extract-low	-7.9839	2.6569	.111	-16.6999	0.7321
	gem+extract-high	-10.0903*	2.6569	.010	-18.8063	-1.3743
	cisplatin+extract-low	-10.3715*	2.6569	.007	-19.0876	-1.6555
	cisplatin+extract-high	-11.8472*	2.6569	.001	-20.5632	-3.1312
cisplatin	gemcitabine	-24.3804*	2.6569	.000	-33.0964	-15.6643
	extract-low	-32.4905*	2.6569	.000	-41.2066	-23.7745
	extract-high	-34.1269*	2.6569	.000	-42.8429	-25.4109
	gem+extract-low	-32.3643*	2.6569	.000	-41.0803	-23.6482
	gem+extract-high	-34.4706*	2.6569	.000	-43.1867	-25.7546
	cisplatin+extract-low	-34.7519*	2.6569	.000	-43.4679	-26.0359
	cisplatin+extract- high	-36.2276*	2.6569	.000	-44.9436	-27.5116
extract-low	gemcitabine	8.1102	2.6569	.097	-0.6059	16.8262
	cisplatin	32.4905*	2.6569	.000	23.7745	41.2066
	extract-high	-1.6364	2.6569	1.000	-10.3524	7.0797
	gem+extract-low	0.1263	2.6569	1.000	-8.5898	8.8423
	gem+extract-high	-1.9801	2.6569	1.000	-10.6961	6.7359
	cisplatin+extract-low	-2.2614	2.6569	1.000	-10.9774	6.4547
	cisplatin+extract- high	-3.7371	2.6569	1.000	-12.4531	4.9790
extract-high	gemcitabine	9.7465*	2.6569	.015	1.0305	18.4626
	cisplatin	34.1269*	2.6569	.000	25.4109	42.8429
	extract-low	1.6364	2.6569	1.000	-7.0797	10.3524
	gem+extract-low	1.7626	2.6569	1.000	-6.9534	10.4787
	gem+extract-high	-0.3438	2.6569	1.000	-9.0598	8.3723
	cisplatin+extract-low	-0.625	2.6569	1.000	-9.3410	8.0910
	cisplatin+extract- high	-2.1007	2.6569	1.000	-10.8167	6.6153
gem+extract-low	gemcitabine	7.9839	2.6569	.111	-0.7321	16.6999
	cisplatin	32.3643*	2.6569	.000	23.6482	41.0803
	extract-low	-0.1263	2.6569	1.000	-8.8423	8.5898
	extract-high	-1.7626	2.6569	1.000	-10.4787	6.9534
	gem+extract-high	-2.1064	2.6569	1.000	-10.8224	6.6096
	cisplatin+extract-low	-2.3876	2.6569	1.000	-11.1037	6.3284
	cisplatin+extract- high	-3.8633	2.6569	1.000	-12.5793	4.8527
gem+extract-high	gemcitabine	10.0903*	2.6569	.010	1.3743	18.8063
	cisplatin	34.4706*	2.6569	.000	25.7546	43.1867
	extract-low	1.9801	2.6569	1.000	-6.7359	10.6961
	extract-high	0.3438	2.6569	1.000	-8.3723	9.0598
	gem+extract-low	2.1064	2.6569	1.000	-6.6096	10.8224
	cisplatin+extract-low	-0.2813	2.6569	1.000	-8.9973	8.4348
	cisplatin+extract-high	-1.7569	2.6569	1.000	-10.4730	6.9591
cisplatin+extract-low	gemcitabine	10.3715*	2.6569	.007	1.6555	19.0876
	cisplatin	34.7519*	2.6569	.000	26.0359	43.4679
	extract-low	2.2614	2.6569	1.000	-6.4547	10.9774
	extract-hjgh	0.6250	2.6569	1.000	-8.0910	9.3410
	gem+extract-bw	2.3876	2.6569	1.000	-6.3284	11.1037
	gem+extract-high	0.2813	2.6569	1.000	-8.4348	8.9973
	cisplatin+extract-high	-1.4757	2.6569	1.000	-10.1917	7.2403
cisplatin+extract-high	gemcitabine	11.8472*	2.6569	.001	3.1312	20.5632
	cisplatin	36.2276*	2.6569	.000	27.5116	44.9436
	extract-low	3.7371	2.6569	1.000	-4.9790	12.4531
	extract-hjgh	2.1007	2.6569	1.000	-6.6153	10.8167
	gem+extract-low	3.8633	2.6569	1.000	-4.8527	12.5793
	gem+extract-high	1.7569	2.6569	1.000	-6.9591	10.4730
	cisplatin+extract-low	1.4757	2.6569	1.000	-7.2403	10.1917

*. The mean difference is significant at the 0.05 level, Sig.: P value

Table 4. The effect on the cytotoxicity of extract modification and concentration.

.

Dependent variable: cytotoxicity					
Source	Type III sum of squares	df	Mean Square	F	P value
Corrected Model	4.3 (a)	8	0.5	62.3	<0.001
Intercept	20.3	1	20.3	2372.3	<0.001
Concentration	3.1	2	1.5	178.2	<0.001
Extract modification	0.8	2	0.4	47.5	<0.001
Concentration × Extract modification	0.4	4	0.1	11.8	<0.001

(a) R squared = 0.89 (Adjusted R squared = 0.87)

Discussion

This work clearly shows that the plain onion Um extract is more cytotoxic in a NSCLC cell line than cisplatin, and perhaps gemcitabine, the most active drugs in NSCLC (14,15). Furthermore, the activity may be greatly enhanced by the addition of an antioxidant cocktail. The IC_{50} value of the modified, onion Um extract (patent pending), 0.02 μg/mL, is among the lowest reported so far in NSCLC in vitro studies.

The addition of Um extract to classical chemotherapeutic agents, gemcitabine and cisplatin, also increased the efficacy of these chemotherapeutics. Thus, these findings together indicate that this extract, or compounds isolated from it, deserve testing in NSCLC and also in other cancers, either on its own, or in conjunction with other chemotherapy drugs.

The next step will be the testing of modified, onion Um extract in animal NSCLC models. If confirmed to be effective, then early clinical studies can follow. As NSCLC is common and the outcome is dismal, even a small benefit from any drug may translate into a huge potential benefit for the population, and, in this regard, natural plant resources will continue to be important as potential candidates for drug development. Paclitaxel, another natural product, for example, was first shown to cause microtubule polymerization in 1983, in the same cell line as we used in this experiment, A549 (16). It was in 1987 that the first clinical activity was demonstrated in a phase 1 trial for a patient with NSCLC (17). Twenty-five years after the initial sets of experiments, it is still one of the current standards of treatment in advanced NSCLC (18). We hope, in the coming years, that new natural drugs may start their journeys towards becoming established treatments for NSCLC. Our finding is another step in that journey.

Many questions remain unanswered at this stage: Is the cytotoxicity of Um extract due to one or few compounds in the extract, or the whole extract itself? What is the exact mechanism with which this drug enhances apoptosis in cancer cells? Our ongoing studies will focus on these issues.

In short, this work shows that the Um extract, or its compounds, may have the potential to serve as cancer drugs. Further work will shed light on whether it will be useful in the clinics.

Acknowledgement

We thank John Rose for his review and suggestions on this paper.

References

1. Peto R, Chen ZM, Boreham J. Tobacco-the growing epidemic. Nat Med 1999; 5: 15.

2. Shrump DS, Nasser KA, Henscke Cl. Non-Small Cell Lung Cancer. In Cancer, Principles and Practice of Oncology, Lippincott Williams & Wilkins, Philadelphia 2005; 753-810.

3. Drugstore Museum. www.drugstoremuseum.com/sections/level_info2.php?level_id=91&level=2

4. Tunckok Y, Kozan O, Cavdar C, Guven H, Fowler J. *Urginea Maritima* (Squill) toxicity. J Toxicol Clin Toxicol 1995; 33: 83-6.

5. El Bahri L, Djegham M, Makhlouf M. *Urginea maritima* L (Squill): a poisonous plant of North Africa Vet Hum Toxicol 2000; 42: 108-10.

6. AAIC 2000 Program. www.aaic.org/01progrm2.htm

7. Iizuka M, Warashina T, Noro T. Bufadienolides and a new lignin from the bulbs of *Urginea Maritima*. Chem Pharm Bull 2001; 49: 282-6.

8. Benkeblia N. Free radical scavenging capacity and antioxidant properties of some selected onions and garlic extracts. Brazilian Archives of Biology and Technology 2005; 48: 753-9.

9. Ozturk OH, Bozcuk H, Burgucu D, Ekinci D, Ozdogan M, Akca S et al. Cisplatin cytotoxicity is enhanced with zoledronic acid in A549 lung cancer cell line: preliminary results of an *in vitro* study. Cell Biol Int 2007; 31: 1069-71.

10. Yang Y, LC QJ, Du QY, Yang BH, Lin Rx, Wang SQ. Combined effects of cantide and chemotherapeutic drugs on inhibition of tumor cells' growth *in vitro* and *in vivo*. World J Gastroenterol 2005; 11: 2491-6.

11. Cui YY, Xie H, Qi KB, He YM, Wang JF. Effects of Pinus Massoniata bark extract on cell proliferation and apoptosis of human Hepatoma BEL-7402 cells. World J Gastroenterol 2005; 11: 5277-82.

12. Yildiz M, Celik-Ozenci C, Akan S, Akan I, Sati L, Demir R et al. Zoledronic acid is synergistic with Vinblastine to induce apoptosis in a multidrug resistance protein-1 dependant way. Cell Biol Int 2006; 30: 278-82.

13. Mekhail T, Kaur H, Ganapathi R, Budd GT, Elson P, Bukowski RM. Phase 1 trial of Anvirzel in patients with refractory solid tumours. Invest New Drugs 2006; 24: 423-7.

14. Pujol JL, Barlesi F, Daures JP. Should chemotherapy combination for advanced non- small cell lung cancer be platinum based? A meta-analysis of phase 3 randomised trials. Lung Cancer 2006; 51: 335-45.

15. Le Chevalier T, Scagliotti G, Natale R, Danson S, Rossell R, Stahel R et al. Efficacy of gemcitabine plus platinum chemotherapy compared with other platinum containing regimens in advanced non-small cell lung cancer: a meta-analysis of survival outcomes. Lung Cancer 2005; 47: 69-80.

16. Wehland J, Henkart M, Klausner R, Sandoval IR. Role of microtubules in the distribution of the Golgi apparatus: effect of taxol and microinjected anti-alpha-tubulin antibodies. Proc Natl Acad Sci U S A 1983; 80: 4286-90.

17. Donehower RC, Rowinsky EK, Grochow LB, Longnecker SM, Ettinger DS. Phase I trial of taxol in patients with advanced cancer. Cancer Treat Rep 1987; 71: 1171-7.

18. Blumenschein GR Jr, Khuri FR, von Pawel J, Gatzemeier U, Miller WH Jr, Jotte RM, et al. Phase III trial comparing carboplatin, paclitaxel, and bexarotene with carboplatin and paclitaxel in chemotherapy-naive patients with advanced or metastatic non-small-cell lung cancer: SPIRIT II. J Clin Oncol 2008; 26: 1879-85.