

ORIGINAL ARTICLE

In vitro cytotoxic effect of garlic extract on malignant and nonmalignant cell lines

Tooba Ghazanfari^{1,2}, Roya Yaraee², Batool Rahmati³, Hoda Hakimzadeh¹, Jalaledin Shams⁴, and Mohammad-Reza Jalali-Nadoushan⁵

¹Immunoregulation Research Center, Shahed University Tehran, I.R., Iran, ²Department of Immunology, Shahed University, Tehran, Iran, ³Department of physiology, Shahed University, Tehran, Iran, ⁴Department of Oncology and Hematology, Shahed University, Tehran, Iran, and ⁵Department of Pathology, Shahed University, Tehran, Iran

Abstract

Cancer is one of the major causes of death in the world and despite many years of research, the treatment of cancer is still a problem. Epidemiological observations and laboratory studies have indicated anticarcinogenic potential of garlic, which has been traditionally used for various human diseases around the world. In this study the cytotoxicity of garlic extract against three malignant cancer cell lines including gastric (AGS), breast (MCF-7) and colon (HT-29) and a nonmalignant cell line (L929) were evaluated by the MTT assay.

The results of this study reveal MCF-7 and AGS cells were sensitive to garlic extract. Despite a many reports on inhibitory effects of garlic on cancer cell line, our data showed these effects are tumor specific and dose dependent. Further studies on animal models and humans are needed to clarify the important molecules and their mechanisms.

Keywords: Garlic, cytotoxic effect, gastric cancer, breast cancer, colon cancer

Introduction

Cancer is a major health problem in the world, and there is not any effective treatment for cancer without side effects up to now. Therefore, research and development of novel and more effective anticancer drugs is necessary. Investigations have shown that a high consumption of fruit and vegetables reduce risk of cancer;⁽¹⁾ moreover, traditionally natural products were frequently used to prevent and treat many diseases, including cancer.

Garlic (*Allium sativum*) belongs to the vegetables of the *Allium* genus. It has traditionally been used as a medicinal plant in the treatment and prevention of a number of diseases, including cancer, coronary heart disease, hypercholesterolemia, obesity and hypertension.^(2–4) Immunomodulatory effects of garlic have been reported in our previous publications.^(5,6) There are also reports on anticarcinogenic and tumor-preventing effects of garlic extract and its components.^(7–9) Garlic, therefore, is a most promising plant for discovery of novel biological active

substances especially anticancer drugs. For this purpose the search of which cancer is influenced by garlic the most and the effective dose with least toxicity on normal and nonmalignant cells is necessary. In our recent findings, we showed that garlic and its fractions are effective on Sk-mel3 melanoma cell line and it could be a very promising chemotherapeutic agent in melanoma treatment.⁽¹⁰⁾ Our results have shown that R100 fraction (molecular weight >100 kDa) of garlic has the most cytotoxic effect in comparison to other fractions. Herein, we investigated the cytotoxic effect of garlic on some other malignant cell lines including breast (MCF-7), gastric (AGS) and colon (HT-29) cancers and a nonmalignant cell line (L929).

Materials and methods

Garlic extraction

Fresh garlic (*Allium sativum* L.) was purchased from Hamadan, Iran. The plant was taxonomically

Address of correspondence: Tooba Ghazanfari, Associate Professor, Department of Immunology, Medical Faculty, Shahed University, Tehran, Iran. PO.Box: 14155-7435. Tel.: +982188964792. Fax: +982188966310. E-mail: tghazanfari@yahoo.com, ghazanfari@shahed.ac.ir

(Received 05 December 2010; revised 28 December 2010; accepted 31 December 2010)

identified by botanists in the herbarium of Department of Pharmacology, Medical Faculty, Shahed University, and voucher specimens of the plant have been deposited. Aqueous garlic extract was prepared by the method used by Ghazanfari et al. Briefly peeled garlic was mixed in the ratio of 1 g of garlic to 1 ml of distilled water, and used as a stock solution.⁽¹¹⁾

Chemicals

RPMI-1640 medium and fetal bovine serum (FBS) were obtained from Gibco. Penicillin-streptomycin was obtained from Sigma-Aldrich. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] powder and phosphate buffer saline (PBS) were obtained from Merck (Germany). Human breast adenocarcinoma (MCF-7) cell line, human gastric adenocarcinoma (AGS) cell line, human colon adenocarcinoma (HT-29) cell line and mouse connective tissue fibroblast (L929) cell line was purchased from the Pasteur Institute, Tehran, Iran.

Cell culture

Malignant and nonmalignant cells were cultured in RPMI-1640 medium (Gibco) containing 10% serum FBS (Gibco) and 100 IU/ml penicillin-streptomycin (Sigma) incubated at 37 °C in a humidified atmosphere; in the presence of 5% CO₂.

Viability assay

MTT reduction assay was used for assessing cells viability. Briefly, MTT powder was dissolved in PBS. Cells were seeded at 10,000/well onto 96-well culture plates and allowed to grow. Then cells were treated with different concentrations (5–0.01 mg/ml) of garlic extract and for different period of times including; 24, 48 and 72 h. Four hours before the end of each period of time 20 µl of MTT

solution (5 mg/ml) was added to each culture. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 h contact period. The supernatants were gently removed and the formazan crystals were resolved in 100 µl acidic isopropanol (0.04 M HCl in isopropanol) and absorbance was read at 540 nm with a plate reader (Stat-Fax 2100).

Statistical analysis

The results are presented as mean ± SEM. Analysis of variation was done and comparisons between study groups were performed with analysis of variance and students t-test. Differences were considered significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

Malignant cells were incubated with various concentrations of garlic extract, then viability of cells measured by MTT assay in 24, 48 and 72 h.

Effect of garlic extract on cell viability of AGS

Our results presented in Figure 1 show that concentrations of 5, 2, 1 and 0.01 of garlic extract significantly ($p < 0.001$) decreased cell viability of AGS cells and concentration of 0.1 mg/ml significantly increased it, at 24 h. Furthermore, cell viability of AGS cells significantly ($p < 0.001$) decreased with concentrations of 5, 2 and 1 mg/ml of garlic at 48 and 72 h (Figure 1).

Effect of garlic extract on cell viability of MCF-7

Cell viability is significantly diminished by concentrations of 5, 2, 1 and 0.1 mg/ml of garlic extract, at 24 h, in addition, concentrations of 5 and 2 mg/ml of garlic extract significantly ($p < 0.05$) decreased cell viability of MCF-7 cells, at 48 h and 72 h (Figure 2).

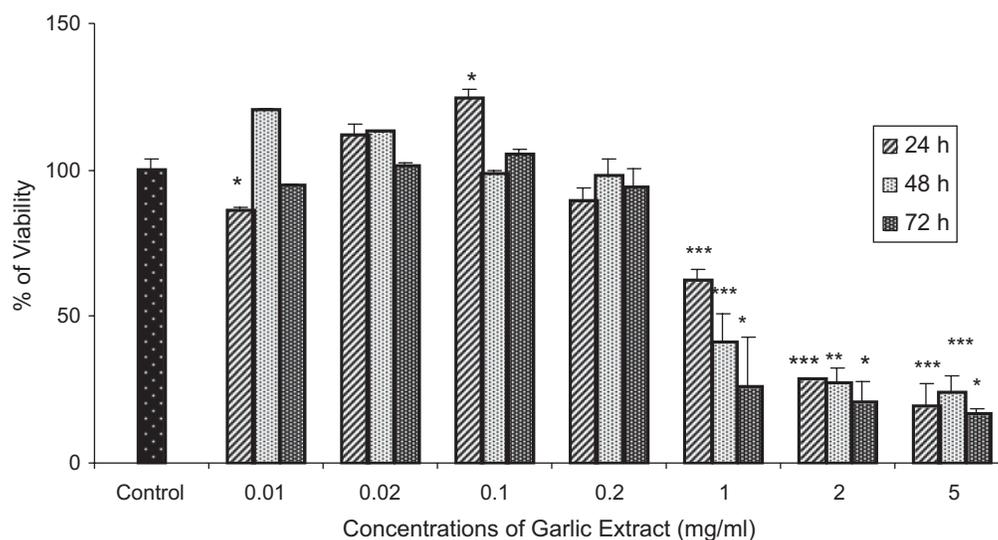


Figure 1. Comparison of viability of different concentrations of garlic extract on AGS cell line with the control group at 24, 48 and 72 h. * denotes significant differences between various concentrations and the control in a corresponding time. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

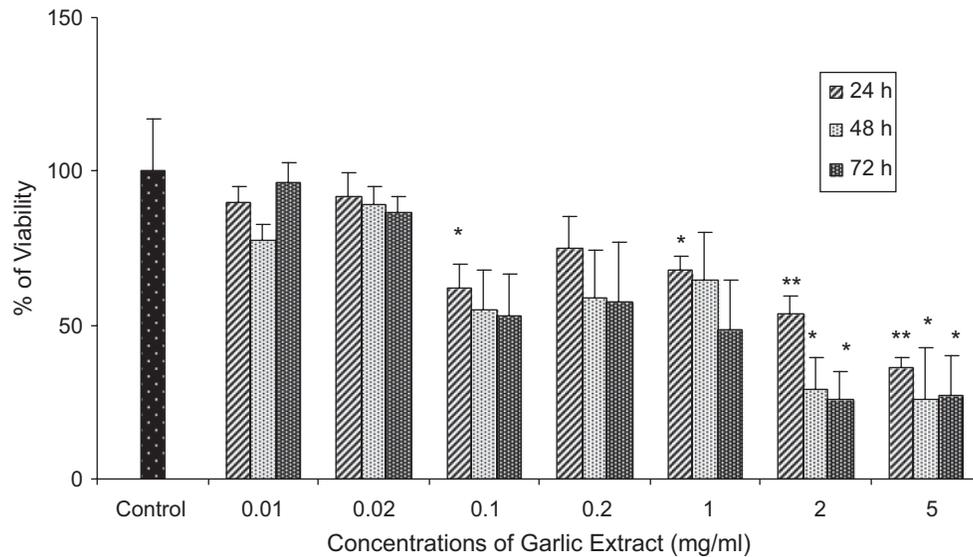


Figure 2. Comparison of viability of different concentrations of garlic extract on MCF-7 cell line with the control group at 24, 48 and 72 h. * denotes significant differences between various concentrations and the control in a corresponding time. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

Effect of garlic extract on cell viability of HT-29

Results presented in Figure 3 shows that after 48 h, cell viability of HT-29 cell line treated by garlic extract in different concentrations 5, 2, 1, 0.2 and 0.01 mg/ml significantly increased. However, garlic extract in the same concentrations after 24 and 72 h could not significantly affect HT-29 cell viability. On the other hand, after 48 h, cell viability increased mainly by high concentrations of garlic extracts (Figure 3).

Effect of garlic extract on cell viability of L929

As shown in Figure 4, garlic extract in concentrations 2, 1 and 0.01 mg/ml increased cell viability of L929 normal cell line after 48 h. Therefore, our results do not show any cytotoxic effect of different concentrations of garlic extract on L929 cell line (Figure 4).

Comparison of cytotoxic effects of garlic extract on malignant (HT-29, MCF-7 and AGS) cell and nonmalignant cells

Results presented in Figure 5 shows that garlic extract significantly decreased cell viability of malignant cells but has not had any cytotoxic effects on nonmalignant cells.

IC₅₀

Doses inducing 50% cell growth inhibition (IC₅₀) against four malignant cells and nonmalignant cells are presented in Table 1.

Discussion

Garlic has traditionally been used as a medicinal plant in many countries and as a therapeutic reagent against various diseases such as cancer.^(2,8) A very extensive literature exists concerning medicinal uses of garlic; thus,

it is a good candidate for the development of anticancer drugs. Various studies indicated protective role of allium vegetables on the risk of several common cancers,⁽¹²⁾ but since it is known that different cell lines might exhibit different sensitivities toward a cytotoxic compound, it is necessary to use of garlic extract on various cell lines. Our previous results has shown that garlic extract induced a significant cytotoxic activity on a melanoma cell line (Sk-mel3), and among garlic fractions R100 and R10 have had more potential in cytotoxic activities against Sk-mel3 melanoma cells.⁽¹⁰⁾ R10 fraction was reported as an immunomodulator that induces cell-mediated immunity in other models such as leishmaniasis.^(5,6,13) Also, the effect of garlic extract and its R10 fraction on macrophage activity has previously been reported.⁽¹¹⁾ Since one of the goals of therapy in cancer treatment is immunotherapy, immunomodulatory effect of garlic along with its cytotoxic effects on cancer cells could make it as a valuable candidate for antitumor drugs. Other studies have shown that components of garlic extract cause reduction in the viability of other melanoma cell lines.^(14,15) In this study, the cytotoxic effect of aqueous garlic extract on AGS, MCF-7, HT-29 and L929 cell lines were investigated.

Gastric cancer

Gastric cancer has remained the second leading cause of cancer death in the world.⁽¹⁶⁾ In our study, the most cytotoxic effect of garlic extract on AGS cell line was achieved by concentration of 5 mg/ml of garlic extract (84%). Allium vegetables (especially garlic and onions) is reported to have cytotoxic effect against gastric cancer.⁽¹⁷⁾ Also allyl trisulfide from garlic is reported to have cytotoxic effect on MGC 803 gastric cell line.⁽¹⁸⁾ Our results confirmed this report but there are some differences between the works. Also, herein, the cytotoxicity

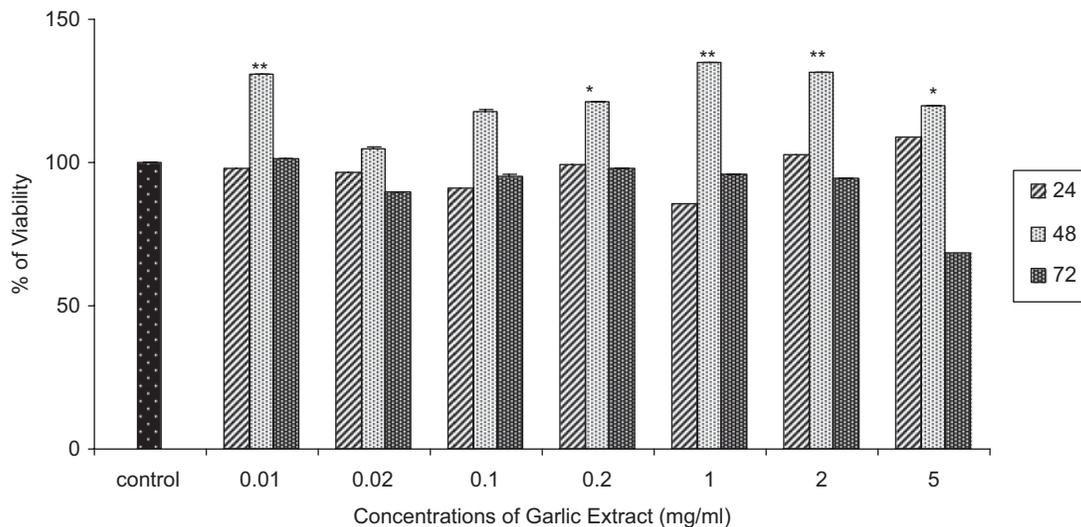


Figure 3. Comparison of viability of different concentrations of garlic extract on HT-29 cell line with the control group at 24, 48 and 72 h. * denotes significant differences between various concentrations and the control in a corresponding time. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

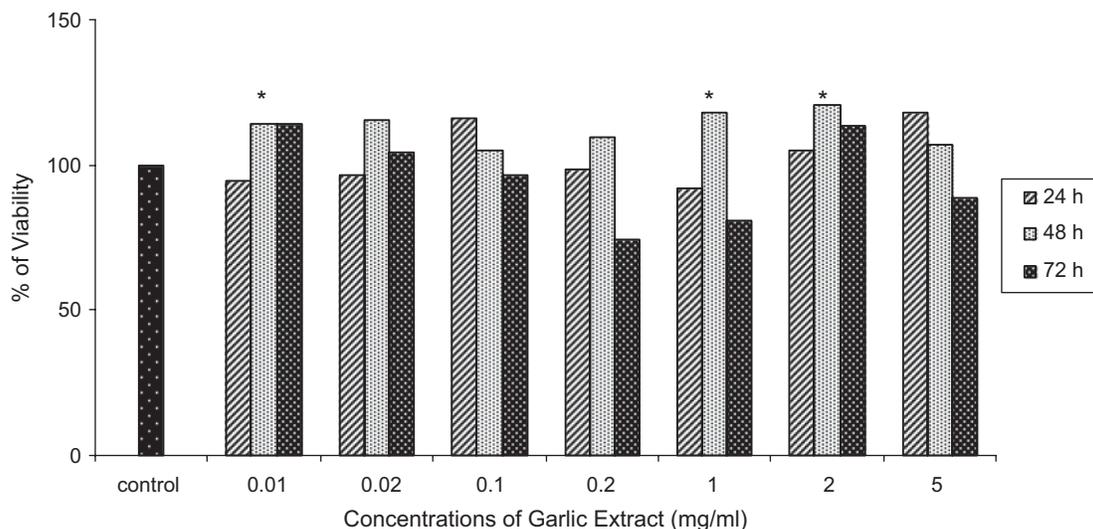


Figure 4. Comparison of viability of different concentrations of garlic extract on L929 cell line with the control group at 24, 48 and 72 h. *denotes significant differences between various concentrations and the control in a corresponding time. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

of garlic extract on AGS cell line is reported for the first time.

Breast cancer

Breast cancer is a worldwide health problem for women.⁽¹⁹⁾ In this study high concentrations of garlic extract induce cytotoxic effect on MCF-7 cells. In 24h culture, concentration of 5 mg/ml has the most cytotoxic effect (75%) on MCF-7 cell line in comparison to other cultured times. Our results are confirmed by some other reports, for instance allium derivatives from garlic are reported to induce significant antiproliferative effects on MCF-7 cell line⁽²⁰⁾ and garlic constituent diallyl trisulfide is reported to induce apoptosis in MCF-7 human breast cancer cells.^(21,22) Altogether, these data confirm the existence of the cytotoxic molecules in the garlic extract against breast cancer.

Colorectal

Colorectal cancer is one of the most common cancers in the world.⁽²³⁾ Our results show that different concentrations of garlic extract has not shown any cytotoxic effects on HT-29 cell line, although high doses of garlic extract caused an increase in viability of HT-29 cell line in comparison with control group at 48h. Dorant et al.⁽²⁴⁾ in an epidemiological study reported no significant inverse association between consumption of garlic supplements and incidence of colon cancer. Our findings confirm this epidemiological study. In contrast, evidence from other studies recommended that protection against colon cancers may be related to consumption of garlic.⁽²⁵⁾ Also, aged garlic extract is reported to suppress proliferation of HT-29 cell line.⁽²⁶⁾ Therefore, it seems that the effect of garlic extract on human colon cancer cell line (HT-29) is

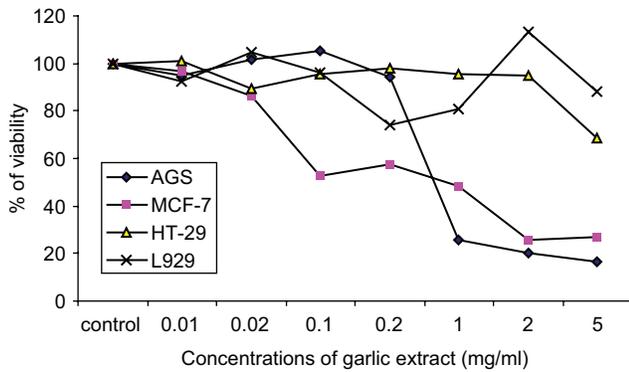


Figure 5. Comparison viability of different concentrations of garlic extract on three malignant cell lines and nonmalignant cell line. * denotes significant differences between various concentrations and the control in a corresponding time. **p*<0.05, ***p*<0.01 and ****p*<0.001 compared to control.

Table 1. Doses inducing 50% cell growth inhibition (IC₅₀) of garlic extract against five cell lines.

	24 h	48 h	72 h
Sk-mel3	4.1 mg/ml	1.675 mg/ml	2.006 mg/ml
AGS	2.488 mg/ml	2.483 mg/ml	2.019 mg/ml
MCF-7	3.087 mg/ml	1.821 mg/ml	1.744 mg/ml
HT-29	>5 mg/ml	>5 mg/ml	>5 mg/ml
L929	>5 mg/ml	>5 mg/ml	>5 mg/ml

different, depending on concentrations, incubation time, kind of garlic extracts and research methodology. Our data show that garlic extract has weak cytotoxic effect on cell viability of HT-29 cells.

IC₅₀

In this study, the IC₅₀ value of garlic extract on malignant (AGS, MCF-7, HT-29) and nonmalignant (L929) cells at 72 h was 2.019 mg/ml, 1.744, >5 mg/ml and 5 mg/ml, respectively. In our previous study, IC₅₀ value of garlic extract on Sk-mel3 cells was 2.006 mg/ml.

Based on our data Sk-mel3, AGS and MCF-7 cells are more than sensitive to garlic in comparison to other cell lines; therefore, garlic is a good candidate for chemotherapeutic drugs on different types of cancers but more investigations on animal models are needed to clarify other mechanisms for cytotoxic and antitumor actions of garlic extract on these cell lines.

Conclusion

The results of this study reveal that despite many reports on inhibitory effects of garlic on cancer cell line proliferation these effects are tumor specific and dose dependent. These findings create a trust for discovery of new drugs with precise cytotoxicity on specific cancer without any toxicity on nonmalignant and normal cells. However, more studies are needed to clarify the exact dose and optimum period of time for garlic antitumor effects on

each kind of cancer and also, more experiments should be done to determine the effective molecule(s) for each cancer.

Acknowledgements

The work of this study was performed by the Immunoregulation Research Center of Shahed University and supported by the Iran National Science Foundation.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Russo, A., Cardile, V., Lombardo, L., Vanella, L., Vanella, A., Garbarino, J.A. Antioxidant activity and antiproliferative action of methanolic extract of *Geum quellyon* Sweet roots in human tumor cell lines. *J Ethnopharmacol* 2005, 100, 323-332.
- Avicenna, A.A. *AP. Al-Qanun Filtibb*. 980-1937, p. 348.
- Aviello, G., Abenavoli, L., Borrelli, F., Capasso, R., Izzo, A.A., Lembo, F., Romano, B., Capasso, F. Garlic: empiricism or science? *Nat Prod Commun* 2009, 4, 1785-1796.
- Iciek, M., Kwiecién, I., Wlodek, L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen* 2009, 50, 247-265.
- Hassan, Z.M., Yaraee, R., Zare, N., Ghazanfari, T., Sarraf Nejad, A.H., Nazori, B. Immunomodulatory affect of R10 fraction of garlic extract on natural killer activity. *Int Immunopharmacol* 2003, 3, 1483-1489.
- Ghazanfari, T., Hassan, Z.M., Ebrahimi, M. Immunomodulatory activity of a protein isolated from garlic extract on delayed type hypersensitivity. *Int Immunopharmacol* 2002, 2, 1541-1549.
- Lamm, D.L., Riggs, D.R. Enhanced immunocompetence by garlic: role in bladder cancer and other malignancies. *J Nutr* 2001, 131, 1067S-1070S.
- Boon, H., Wong, J. Botanical medicine and cancer: a review of the safety and efficacy. *Expert Opin Pharmacother* 2004, 5, 2485-2501.
- Butt, M.S., Sultan, M.T., Butt, M.S., Iqbal, J. Garlic: nature's protection against physiological threats. *Crit Rev Food Sci Nutr* 2009, 49, 538-551.
- Hakimzadeh, H., Ghazanfari, T., Rahmati, B., Naderimanesh, H. Cytotoxic effect of garlic extract and its fractions on Sk-mel3 melanoma cell line. *Immunopharmacol Immunotoxicol* 2010, 32, 371-375.
- Ghazanfari, T., Hassan, Z.M., Khamesipour, A. Enhancement of peritoneal macrophage phagocytic activity against *Leishmania major* by garlic (*Allium sativum*) treatment. *J Ethnopharmacol* 2006, 103, 333-337.
- Galeone, C., Pelucchi, C., Levi, F., Negri, E., Franceschi, S., Talamini, R., Giacosa, A., La Vecchia, C. Onion and garlic use and human cancer. *Am J Clin Nutr* 2006, 84, 1027-1032.
- Ghazanfari, T., Hassan, Z.M., Ebtekar, M., Ahmadiani, A., Naderi, G., Azar, A. Garlic induces a shift in cytokine pattern in *Leishmania major*-infected BALB/c mice. *Scand J Immunol* 2000, 52, 491-495.
- Takeyama, H., Hoon, D.S., Saxton, R.E., Morton, D.L., Irie, R.F. Growth inhibition and modulation of cell markers of melanoma by S-allyl cysteine. *Oncology* 1993, 50, 63-69.
- Ledezma, E., Apitz-Castro, R., Cardier, J. Apoptotic and anti-adhesion effect of ajoene, a garlic derived compound, on the

- murine melanoma B16F10 cells: possible role of caspase-3 and the alpha(4)beta(1) integrin. *Cancer Lett.* 2004, 206, 35-41.
16. Malekzadeh, R., Derakhshan, M.H., Malekzadeh, Z. Gastric cancer in Iran: epidemiology and risk factors. *Arch Iran Med* 2009, 12, 576-583.
 17. Setiawan, V.W., Yu, G.P., Lu, Q.Y., Lu, M.L., Yu, S.Z., Mu, L., Zhang, J.G., Kurtz, R.C., Cai, L., Hsieh, C.C., Zhang, Z.F. Allium vegetables and stomach cancer risk in China. *Asian Pac J Cancer Prev* 2005, 6, 387-395.
 18. Pan, X.Y., Li, F.Q., Yu, R.J., Xie, G.F., Wang, H., Zhao, L.Y., Zhang, Q. [Experimental chemotherapy of human gastric cancer cell lines *in vitro* and in nude mice]. *Zhonghua Zhong Liu Za Zhi* 1988, 10, 15-18.
 19. Stan, S.D., Zeng, Y., Singh, S.V. Ayurvedic medicine constituent withaferin a causes G2 and M phase cell cycle arrest in human breast cancer cells. *Nutr Cancer* 2008, 60(Suppl 1), 51-60.
 20. Pinto, J.T., Rivlin, R.S. Antiproliferative effects of allium derivatives from garlic. *J Nutr* 2001, 131, 1058S-1060S.
 21. Malki, A., El-Saadani, M., Sultan, A.S. Garlic constituent diallyl trisulfide induced apoptosis in MCF7 human breast cancer cells. *Cancer Biol Ther* 2009, 8, 2175-2185.
 22. Lei, X.Y., Yao, S.Q., Zu, X.Y., Huang, Z.X., Liu, L.J., Zhong, M., Zhu, B.Y., Tang, S.S., Liao, D.F. Apoptosis induced by diallyl disulfide in human breast cancer cell line MCF-7. *Acta Pharmacol Sin* 2008, 29, 1233-1239.
 23. Winawer, S.J., Fletcher, R.H., Miller, L., Godlee, F., Stolar, M.H., Mulrow, C.D., Woolf, S.H., Glick, S.N., Ganiats, T.G., Bond, J.H., Rosen, L., Zapka, J.G., Olsen, S.J., Giardiello, F.M., Sisk, J.E., Van Antwerp, R., Brown-Davis, C., Marciniak, D.A., Mayer, R.J. Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology* 1997, 112, 594-642.
 24. Dorant, E., van den Brandt, P.A., Goldbohm, R.A., Hermus, R.J., Sturmans, F. Garlic and its significance for the prevention of cancer in humans: a critical view. *Br J Cancer* 1993, 67, 424-429.
 25. Kim, J.Y., Kwon, O. Garlic intake and cancer risk: an analysis using the Food and Drug Administration's evidence-based review system for the scientific evaluation of health claims. *Am J Clin Nutr* 2009, 89, 257-264.
 26. Matsuura, N., Miyamae, Y., Yamane, K., Nagao, Y., Hamada, Y., Kawaguchi, N., Katsuki, T., Hirata, K., Sumi, S., Ishikawa, H. Aged garlic extract inhibits angiogenesis and proliferation of colorectal carcinoma cells. *J Nutr* 2006, 136, 842S-846S.