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

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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
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% CO\(_2\).  

**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 4h 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 and 72h (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.](image-url)
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.

IC<sub>50</sub>
Doses inducing 50% cell growth inhibition (IC<sub>50</sub>) 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. 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, 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. R10 fraction was reported as an immunomodulator that induces cell-mediated immunity in other models such as leishmaniasis. Also, the effect of garlic extract and its R10 fraction on macrophage activity has previously been reported. 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. 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. 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. Also allyl trisulfide from garlic is reported to have cytotoxic effect on MGC 803 gastric cell line. Our results confirmed this report but there are some differences between the works. Also, herein, the cytotoxicity
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
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.

\[ \text{IC}_{50} \]

In this study, the \( \text{IC}_{50} \) value of garlic extract on malignant (AGS, MCF-7, HT-29) and nonmalignant (L929) cells at 72 h was 2.019 mg/ml, 1.74 mg/ml, >5 mg/ml and 5 mg/ml, respectively. In our previous study, \( \text{IC}_{50} \) 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.

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**Declaration of interest**

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

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