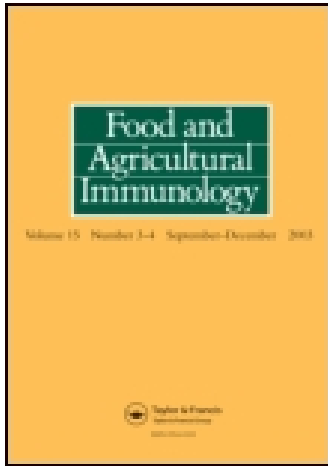


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Cytotoxic effect of four herbal medicines on gastric cancer (AGS) cell line

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Gastric cancer (GC) is the fourth most common cancer and second leading cause of cancer-related death worldwide. Therefore, discovery of novel anti-cancer herbal drugs is of importance. Herbal medication is now being used for treatment of various diseases, including cancer in many countries. In this study, the cytotoxic effect of traditional herbal medicines (Aloe vera, Ginger, *Ziziphora* and Saffron extracts) was investigated on gastric AGS cell line. MTT assay was performed for cytotoxic effect of these herbal medicines. The most cytotoxic effects of Ginger, *Ziziphora* and Saffron extracts on AGS cell line were 47, 88 and 67%, respectively, compared to control group. According to our data, among these herbal extracts, *Ziziphora* has the highest cytotoxic effect on AGS cell line. *Ziziphora* extract seems to be a good candidate as an anti-cancer agent against GC. To clarify the effective molecules and their mechanisms, further studies are undertaken on animal models and humans.

Keywords: gastric cancer (AGS); Aloe vera; Ginger; Saffron; *Ziziphora*; cytotoxic

1. Introduction

Gastric cancer (GC) is the fourth most common form of malignant diseases in the worldwide (Tepes, 2009; Tsugane & Sasazuki, 2007). Because GC prognosis is poor and it is often diagnosed in locally advanced or metastatic stages, its treatment remains a great challenge (Tetzlaff, Cen, & Ajani, 2008; Zaniboni & Meriggi, 2005).

Herbal medicines are rich sources of natural anti-cancer materials (Tan & Vanitha, 2004) and are thus good candidates for the development of anti-cancer drugs. In an attempt to identify novel herbal anti-cancer agents from traditional herbal medicines, we studied the in vitro cytotoxic effects of the Aloe vera, Ginger, *Ziziphora* and Saffron extracts on adenocarcinoma gastric cell line (AGS).

Aloe vera, a member of the family *Liliaceae*, is used as a natural treatment for various types of diseases such as cancer (El-Shemy et al., 2010; Vega, Uribe, Lemus, & Miranda, 2007).

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Ginger, the rhizome of *Zingiber officinale*, is one of the most widely used species of the Ginger family (*Zingiberaceae*) which exhibits anti-oxidant, anti-inflammatory, anti-fungal and anti-carcinogenic properties (Surh, 2002; Surh, Lee, & Lee, 1998). According to our knowledge, there are no more studies regarding cytotoxic effect of Ginger extract on AGS cell line (Ishigura et al., 2007).

Since ancient times, Saffron (*Crocus sativus* L.) has been used in traditional medicine for various ailments (Abdullaev & Espinosa-Aguirre, 2004; Chryssanthi et al., 2007). Cytotoxic effect of Saffron extract has been studied in some cancer cell lines (Abdullaev, 2002, Abdullaev et al., 2003; Goel & Aggarwal, 2010), but it has not been investigated on AGS cell line, up to now.

Ziziphora (*Z. clinopodioides* Lam), a traditional Uygur medicinal plant is used for the treatment various diseases (Salehi, Sonboli, Eftekhari, Nejad-Ebrahimi, & Yousefzadi, 2005; Tian, Shi, Yu, & Upur, 2010). According to our knowledge, this is the first study of the effects of an aqueous extract of *Ziziphora* on cell viability of cancer cell line.

Herein, we investigated the cytotoxic effect of the extracts of these four herbal medicines on AGS gastric cancer cell line.

2. Materials and methods

2.1. Reagents

RPMI-1640 medium and foetal bovine serum (FBS) were obtained from Gibco. Penicillin–streptomycin was obtained from Sigma-Aldrich, USA. MTT [3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] powder and phosphate buffer saline (PBS) were obtained from Merck (Germany).

2.2. Plant materials

To prepare the extracts, the valuable part of each plant in traditional medicine was used. The gel of Aloe vera was supplied by Aloe vera Co. (Bushehr, Iran). Stigma of Saffron was collected from Mashhad (Iran), and aerial parts of *Ziziphora* and rhizomes of Ginger were supplied by local market (Tehran, Iran). These plants were then processed in Pharmacological Research Centre of Medicinal Plants, Shahed University.

2.3. Preparation of herbal extracts

In the present study, total aqueous herbal extracts were prepared by mixing with distilled and deionised water and centrifuged (5000 g/30 min), and the resultant supernatant was collected and stored in refrigerator until used. All extracts were used in different concentrations (5, 2, 1, 0.2, 0.1, 0.05, 0.02 and 0.01 mg/ml) based on their weight (mg) per water volume (ml).

2.4. Cell culture

Adenocarcinoma gastric cell line (AGS) was purchased from Pasteur Institute (Tehran, Iran) and maintained in RPMI with 10% FBS (both of them from Gibco) incubated at 37°C and 5% CO₂. Exponentially growing AGS cells were digested by

2 ml trypsin 0.25% for 1–2 min. RPMI 1640 medium containing 10% FBS was subsequently added. Final cell suspensions were placed in 96-well plates ($2 \times 10^4/200 \mu\text{l}/\text{well}$) in an incubator containing 5% CO_2 and incubated at 37°C for 24 h. Then, $200 \mu\text{L}$ RPMI 1640 medium containing different concentrations (5–0.01 mg/ml) of each herbal extract (Aloe vera, Ginger, Saffron and *Ziziphora*) were added to each well of the plate. Cells were cultured for 24, 48 and 72 h.

2.5. Cell viability

We used MTT reduction assay for evaluating cells viability. Briefly, MTT powder (Merck, Germany) was dissolved in PBS (5 mg/ml). Cells were seeded at 20,000/well onto 96-well culture plates and allowed to grow. Then, the cells were treated with different concentrations of each herbal extract for 24, 48 and 72 hours. Four hours before reading of absorbance, 20 ml of MTT solution was added to each culture and cells were incubated for 4 h with MTT solution. Subsequently, MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4 h contact period. The supernatants were then gently removed, the formazan crystals were resolved in $100 \mu\text{l}$ acidic isopropanol (0.04 M HCl in isopropanol), and absorbance was read at 540 nm with a plate reader.

2.6. Statistical analysis

The results are presented as mean \pm SEM. Analysis of variation was done and comparisons between study groups were made with ANOVA and student's *t*-test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of Aloe vera extract on cell viability of AGS cell line

The results illustrated in Figure 1 show that there are no significant differences between test group and control group.

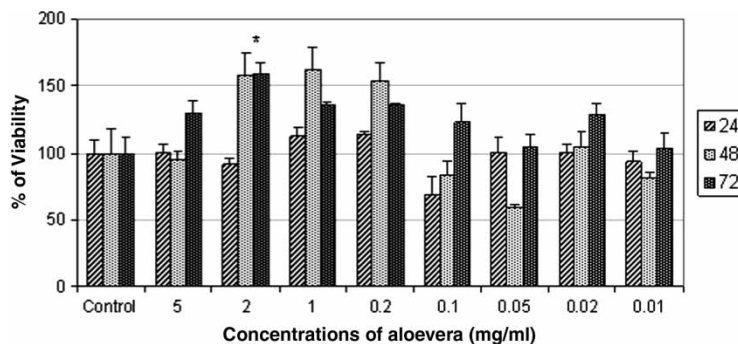


Figure 1. Effect of aqueous extract of Aloe vera on cell viability of AGS cell line at 24, 48 and 72 h. The Aloe vera extract showed no significant cytotoxic activity against AGS gastric cancer cell line. * denotes significant differences compared to control group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

3.2. Effect of Ginger extract on cell viability of AGS cell line

As our results show, cells treated with concentrations of 5, 0.1 and 0.01 mg/ml significantly decreased cell viability of AGS cells at 24, 48 and 72 h, whereas doses of 2 and 1 mg/ml significantly increased cell viability in comparison with control group at three times (Figure 2).

3.3. Effect of Ziziphora extract on cell viability of AGS cell line

According to our results, cells treated with concentrations of 5, 2 and 1 mg/ml significantly ($p < 0.01$) decreased cell viability of AGS cells at 48 and 72 h, and doses of 5 and 2 mg/ml significantly ($p < 0.05$) decreased cell viability. However, no significant differences were observed compared to the control group (Figure 3).

3.4. Effect of Saffron extract on cell viability of AGS cell line

The results presented in Figure 4 show that cell viability of AGS cell was significantly decreased by high concentration (5 mg/ml) of Saffron extract at 24, 48 and 72 h, whereas other concentrations of Saffron extract showed no cytotoxic effects on AGS cell line in 24, 48 and 72 h.

3.5. Comparison of cytotoxic effects of the four herbal extracts on AGS cell line

Our data suggest that *Ziziphora* extract is effective on AGS cells line higher than other extracts. Concentrations inducing 50% cell growth inhibition (IC_{50}) on AGS cells are provided in Table 1.

4. Discussion

GC remains one of the most common causes of cancer-related deaths worldwide (Milano et al., 2006). Poor prognosis (Zhao et al., 2008) and side effects of current anti-cancer drugs in the advanced GC are still the major problems for GC treatment.

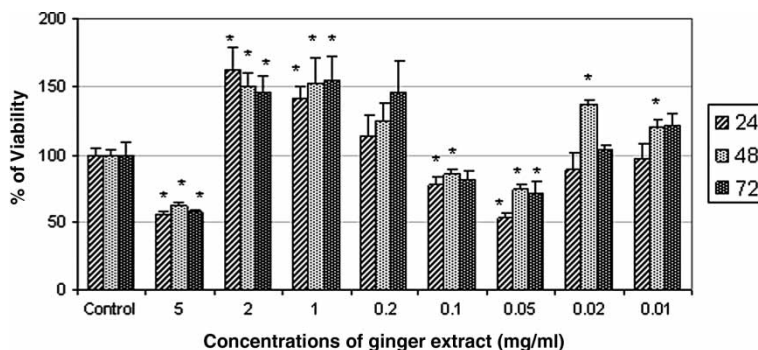


Figure 2. Effect of aqueous extract of Ginger on cell viability of AGS cell line at 24, 48 and 72 h. The highest cytotoxic effect of Ginger extract on AGS cell line in 24 h was achieved at dose of 0.05 mg/ml. * denotes significant differences compared to control group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

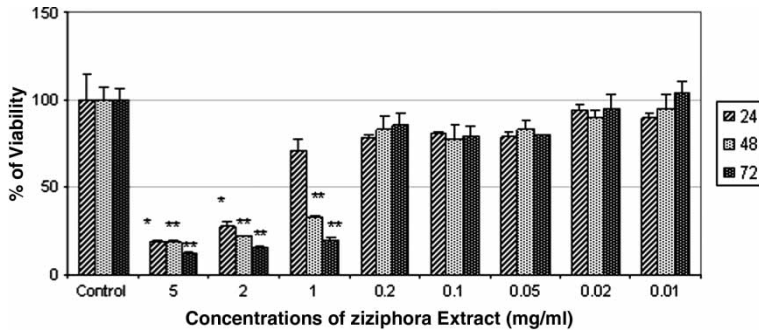


Figure 3. Effect of aqueous extract of *Ziziphora* on cell viability of AGS cell line at 24, 48 and 72 h. The highest cytotoxic effect of *Ziziphora* extract on AGS cell line in 72 h was achieved at dose of 5 mg/ml. * denotes significant differences compared to control group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

In the recent years, many studies have focused on using herbal medicines. Main advantage of natural products is their mild side effects in comparison with chemical drugs. Therefore, discovery of novel anti-cancer herbal drugs is of importance. The aim of this study was to investigate the cytotoxic effects of Aloe vera, Saffron, Ginger and *Ziziphora* extracts on AGS gastric cell line.

4.1. Aloe vera extract

Our findings showed that, the Aloe vera extract did not show any significant cytotoxic activity on AGS gastric cancer cell line. In the previous studies, cytotoxic effect of Aloe vera extract has been evaluated on various GC cell lines. Chen, Lin, Chang, Fang and Lin (2007) reported that aloe-emodin exhibits an anti-cancer effect against gastric AGS and NCI-N87 cell lines (Chen et al., 2007). Our finding is in disagreement with those of Chen, it seems that the effect of Aloe vera extract on

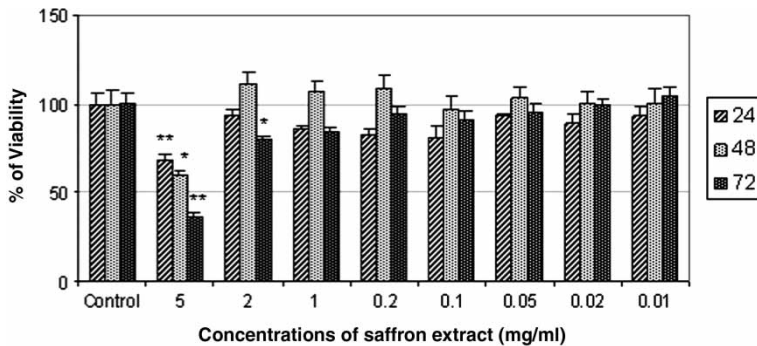


Figure 4. Effect of aqueous extract of Saffron on cell viability of AGS cell line at 24, 48 and 72 h. The highest cytotoxic effect of Saffron extract on AGS cell line in 72 h was achieved at dose of 5 mg/ml. * denotes significant differences compared to control group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control.

Table 1. Concentrations inducing 50% cell growth inhibition (IC₅₀) of Aloe vera, Ginger, *Ziziphora* and Saffron extracts against AGS cell line.

IC50	24 h	48 h	72 h
<i>Ziziphora</i> (mg/ml)	2.356	1.779	1.674
Ginger (mg/ml)	> 5	> 5	> 5
Saffron (mg/ml)	2.95	2.064	3.96
Aloe vera (mg/ml)	> 5	> 5	> 5

human gastric cancer cell line (AGS) is different, depending on concentrations incubation and kind of Aloe vera extracts.

4.2. *Ginger extract*

Regarding the exposure of AGS cell line to Ginger, the highest cytotoxic effect of Ginger on AGS cell line in 24, 48 and 72 h was achieved at dose of 0.05 mg/ml (47%). Ishiguro et al. (2007) reported that 1–4 µg/ml of 6-Shogaol (another ingredient of Ginger) reduced cell viability of AGS, HGC and KATO III gastric cancer cell lines at 24 h (Ishiguro et al., 2007). Although applied doses and materials used in Ishiguro's study are different from those in our study, that data confirmed the present work.

4.3. *Ziziphora extract*

In the present study, our results show that the most cytotoxic effect of *Ziziphora* on AGS cell line in 72 h was achieved at dose of 5 mg/ml (88%). To our knowledge, no previous report has been published on the anti-cancer effect of *Ziziphora* on cancer cell lines, up to now.

4.4. *Saffron extract*

In the last few years, Saffron has been known for its anti-cancer and anti-tumour properties, and its cytotoxic effects have been studied on some cancer cell lines (Abdullaev, 2002; Abdullaev et al., 2003). So far, there has been no report concerning cytotoxic effect of Saffron extract on various gastric cell lines. In the present study, dose of 5 mg/ml of Saffron extract had the highest cytotoxic effect on AGS cell line at 72 h (64%). Regarding our data, this study shows that AGS cells are more sensitive to *Ziziphora* than the other three herbal extracts (Table 1).

These results suggest that *Ziziphora* extract is a chemotherapeutic drug candidate for treatment of GC. Further studies in animal models are needed to clarify other mechanisms for cytotoxic and anti-tumour actions of *Ziziphora* on AGS cell line and on GC in humans.

Acknowledgements

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