Effect of Saffron on the Viability of Normal and Malignant Human Cells In Vitro

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Abstract

Saffron, obtained from the dried stigmas of Crocus sativus L., is an important spice, rich in carotenoids. Carotenoids are capable of protecting cells from the attack of free radicals, which naturally develop during sunbathing, smoking, exercise – even breathing oxygen. It has been estimated that our body needs 6 to 30 mg of carotenoids per day to protect its cells. These amounts are normally consumed as fruits, vegetables and agents for flavoring and coloring foods in different parts of the world. We have investigate the effect of aqueous extracts of saffron upon cell viability of different human cells, including normal human lung fibroblasts and several human malignant cell lines (MCF-7, SKNH and HeLa), using an assay based on tetrazolium dye. Cell lines were seeded into 96-well plates at 10X10³ cell per well and exposed during 18 h with saffron extracts. We observed that, while saffron extracts produced no changes in cell viability of normal human lung fibroblast, a dose-dependent inhibitory effect was observed on malignant cells. We found that MCF-7 cells were the more sensitive ones, followed by SKNM and HeLa cells (IC₅₀ (mg/ml) were for MCF-7 –0.78; SKNM – 1,66, HeLa – 1,92 and normal cells –19,99).

INTRODUCTION

From ancient times, saffron, which harvested from the dried, dark red stigmas of Crocus sativus L. flowers, has been used as a drug to treat various human health conditions such as coughs, flatulence, stomach disorders, colic, insomnia, chronic uterine haemorrhage, amenorrhea, dysmenorrhoea, feminine disorder, scarlet fever, smallpox, colds, insomnia, asthma and cardiovascular disorders (Winterhalter and Straubinger, 2000; Abdullaev, 2003). Historical records detailing the use saffron go back to ancient Egypt and Rome where it was used as a dye in perfume and as a spice for culinary purposes. Currently, saffron applies the characteristic flavour and colour Spanish paella, Italian risotto, French bouillabaisse, Mexican fiambre, Arabic lamb and chicken dishes, Iranian plov, Azerbaijani pakhlava, Indian dessert sauces, as well as Swedish, Cornish and Pennsylvania Dutch holiday breads. Saffron has also been used in cosmetic industry (Szita, 1987; Abdullaev, 2002).

Characteristic ingredients of saffron are colouring components, carotenoids; a bitter taste, picrocrocin and a spice aroma, safranal (Abdullaev, 1993; Tarantilis et al., 1995) the beginning of 90’s it was for the first time reported that saffron extract inhibited the growth of malignant cells in vivo and in vitro (Nair et al., 1991; Abdullaev and Frenkel, 1992). During the last decade, a number of studies in animal and model systems have demonstrated an antitumour effect of saffron and its constituents on different malignant cells which was discussed in my recent review (Abdullaev, 2002). Saffron had a dose-dependent inhibitory effect on carcinoma, sarcoma, leukaemia and several other malignant cells in the test tube. Saffron increased the life span of treated tumour-bearing mice compared to untreated animals by 45-120 percent (Nair et al, 1991). Different hypothesis for the anticancerogenic and antitumour effects of saffron and its ingredients...
have been proposed including the inhibition of nucleic acid and free radical chain reactions, interaction of carotenoids with topoisomerase II. It was also reported that saffron is non-toxic and had no effect on the growth of normal cells (Abdullaev, 2002, 2003). It was reported (Tarantilis et al., 1994; Escribano et al., 1996) that saffron crude extract and some of its isolated compounds inhibited the growth of human malignant cells in vitro. In view of renewed interest in plant agents and cancer, both in the scientific and medical communities, it would be interesting to examine the cytotoxic effect of saffron extract on human normal and malignant cells in model system. The aim of present work we studied the effect of saffron extract from four countries on the viability of human normal and malignant cells in vitro.

MATERIALS AND METHODS

Materials
The concentrated saffron extracts of different regions (Azerbaijan, Spain, Iran, and India) were prepared. And saffron stock solutions of 4 mg/mL were prepared in culture medium, protected from light and keep at -20°C until use. Dilutions were prepared in culture medium immediately prior to use. Cell culture media and serum were obtained from Gibco-BRL, Inc. (Gaithersburg, MD)

Cell Cultures
The cell lines utilized were SKNSH (malignant cells derived from a bone metastases of a neuroblastome), HeLa (malignant cells from an adenocarcinoma from the uterine cervix), MCF-7 (malignant cells from a breast tumor), and normal fetal lung fibroblast cells. All the cells lines are of human origin, and they were obtained from ATCC (American Type Culture Collection). The cell lines were routinely maintained as a monolayer in Dulbecco’s modified Eagle’s medium (DEMEM) supplemented with 10% FCS (fetal calf serum) (Gibco BRL, Gaithersburg, MD), and incubated at 37°C in 5%CO2–95% air at high humidity. Cells were harvested with 0.025% trypsin (Sigma St. Louis, MO) and 0.01% EDTA (Gibco BRL, Gaithersburg, MD).

Growth Inhibition Experiments
The effect of Spanish saffron extract on cell viability of SKNSH, HeLa, MCF-7 and lung fibroblastos; and the effect of saffron extracts of different regions (Azerbaijan, Spain, Iran, and India) on cell viability in SKNSH cells was evaluated using the XTT assay (sodium 3’-[1-(phenylamino-carbonyl)-3,4-tetrazolium]–bis) (Roche Molecular Biochemical. The assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolic active cells. The system was performed as follows: cells were each seeded into 96-well plates (Costar Cambridge, Massachusetts, USA) at density 10x10^3 viable cell/well in 100 µl culture medium. After 24 h, the culture medium was removed and fresh medium with various amounts of saffron extracts (0, 0.25, 0.5, 1.0, 2.0 and 4 mg/mL) were added, the cells were cultivated for 18 h, and cell viability was evaluated using the XTT-assay. Briefly, when the saffron protocol finished, was added to each well 50 µl of XTT (final concentration 0.3 mg/ml) and incubated for 4 h in a humidified atmosphere (37°C, 5% CO2). The measure of the samples spectrophotometrical absorbance was using a microtiter plate ELISA reader at 492 nm.

Determination of IC50
The mean concentration in each set of 3-4 wells was measured by triplicate. The percentage of growth inhibition was calculated and IC50 values (concentration of drug to achieve 50% growth inhibition) were obtained graphically from the survival curves distribution.

RESULTS AND DISCUSSION
The effect of aqueous solution of Spanish saffron on viability of four malignant
and normal human cell lines was evaluated. SKNSH (malignant cells derived from a bone metastases of a neuroblastome), HeLa (malignant cells from an adenocarcinoma from the uterien cervix), MCF-7 (malignant cells from a breast tumor) and human normal lung fibroblasts were included in this study. The effect of saffron was evaluated as indicated in Material & Methods, and results are shown in the Figures 1, 2, 3, and 4 respectively. Data obtained clearly indicate that Spain saffron extract inhibit cell viability of all malignant cells in a dose-dependent manner. There was no significant effect of saffron on cell viability of normal human lung fibroblasts, at none dose. IC$_{50}$ (concentration needed to inhibit 50% growth) are shown in Table 1. These data indicated that IC$_{50}$ for normal cells were more 10 times higher than for tested human malignant cells.

Results of these experiments clearly indicated that saffron extract inhibited the viability of different human malignant cells in vitro, and had no effect at tested concentrations of saffron on human normal cells. We obtained additional evidence that supported previous data about selective inhibitory effect of saffron on malignant cells (Abdullaev and Frenkel, 1992a,b; Nair et al., 1995). At present, the molecular mechanism of inhibitory action of saffron against malignant cells are not clear, although different hypothesis have been put forward (Abdullaev, 2002). More detail investigations on antitumour and anticancer effects of saffron and its main components in model and animal systems will allow to suggest that this natural products can be use in clinical trials as cancer chemopreventive agents.

Literature Cited
Tables

Table 1. Concentrations of Spanish saffron extract necessary to inhibit 50% of cell growth

<table>
<thead>
<tr>
<th>Cells</th>
<th>SKNSH</th>
<th>HeLa</th>
<th>MCF-7</th>
<th>Normal Lung Fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI50 (mg/mL)</td>
<td>1.66</td>
<td>1.92</td>
<td>0.78</td>
<td>19.99</td>
</tr>
</tbody>
</table>

Figures

Fig. 1. Effect of saffron on viability of normal human cells.
Fig. 2. Effect of saffron on viability of HeLa human malignant cells.
Fig. 3. Effect of saffron on viability of MCF-7 human malignant cells.
Fig. 4. Effect of saffron on viability of SKNSH human malignant cells.