Cytotoxic Potential of Antioxidants from Tomatoes on Tumoral Cells

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Abstract

Tomatoes (*Lycopersicon esculentum Mill.*) are known to contain various antioxidants such as lycopene and other carotenoids, which were proven to have antineoplastic activity. The aim of the present study was to obtain and characterize a whole tomato extract in order to show the amount and type of antioxidants contained, as well as to assess the cytotoxic potential of such extracts on the HepG2 tumoral cell line. Tomato extracts were obtained using the light petroleum/ethyl acetate/methanol method and characterized by HPLC. HepG2 liver hepatocellular carcinoma cell line was grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS. As soon as subconfluency was reached cells were transferred into 96 well plates and treated with serial dilutions of the tomato extract. Cytotoxicity of the extracts was assessed using the MTT dye, in comparison with untreated cells and IC50 was established. The extraction method proved to be a very efficient one, yielding significant amounts of carotenoid pigments, including lycopene, β-carotene, γ-carotene and others, as shown by the HPLC analysis. The antioxidants contained by the extracts showed significant cytotoxicity on HepG2 liver hepatocellular carcinoma cell line, being able to visibly inhibit cellular development in vitro. The present study showed the cytotoxic influence that antioxidants extracted from tomatoes exert on the in vitro development of a hepatocellular carcinoma cell line, opening new perspectives for in vivo studies, involving patients with hepatic cancer.

Keywords: *antioxidants, carotenoids, cytotoxicity, tumoral cells*

INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver. It is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected (Cicalese, 2014). *In vitro* animal and clinical studies suggest that lycopene, a nonprovitamin A carotenoid and a potent antioxidant, may attenuate the liver injury and possibly prevent the development of HCC (Seren *et al.*, 2008). Tomatoes (*Lycopersicon esculentum Mill.*) contain plenty of lycopene, together with other antioxidants like ascorbic acid, α-tocopherol, β-carotene and lutein (Kotkov *et al.*, 2011) which were proven to have antineoplastic activity. HepG2 is a stable human tumour cell line derived from a liver hepatocellular carcinoma of a 15 year old

Caucasian male. It has been used extensively for *in vitro* studies and is particulary suitable for cytotoxicity models (Dai *et al.*, 1993; Scolastici *et al.*, 2008; Xu *et al.*, 2003).

The aim of the present study was to obtain and characterize a whole tomato extract in order to show the amount and type of carotenoid antioxidants contained, as well as to assess the cytotoxic potential of such extracts on the HepG2 tumoral cell line.

MATERIALS AND METHODS

Fully matured Menhir F1 tomatoes (*Lycopersicon esculentum Mill.*) were used in order to obtain the extract. They had been cultivated in greenhouse tunnels, respecting all requirements of organic farming. Extraction was performed three times with light petroleum/ethyl acetate/ methanol (1:1:1, v/v/v) respecting the protocol described by Breithaupt and Schwack (2000). After addition of water and phase separation, the extract was filtered, evaporated and submitted to high performance liquid chromatography (HPLC) analysis, using a Shimadzu LC20 AT equipment with a SPD-M20A diode array detector and an YMC C30 column (24 cm x 4.6 mm, 5 µm). The mobile phases were represented by methanol and methyl-tert-butyl ether (MTBE). The DAD detector was set at 470 nm and the flow rate was 1 ml/min. The retention time as well as UV-Vis spectrum of each carotenoid was compared to standard solutions (LGC Standards, UK). Concentration assessment was performed using a calibration curve obtained after plotting peak area versus concentration for five different concentrations of the standards, between 1-50 µg/ml.

Human HepG2 liver hepatocellular carcinoma cell line was obtained from European Collection of Cell Cultures (ECACC, Salisbury, UK) and grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS (Sigma Aldrich, St Louis, MO, USA). Cells were trypsinized at subconfluency, resuspended in culture medium and transferred into 96-well plates (Nunclon, Thermo Fischer Scientific, Waltham, MA, USA), at a density of $15x10^3$ cells/well. Each well contained 190 µl of culture media. Cells were incubated at 37°C, 5% CO2 and saturated humidity for 24 hours, until attachment occurred. At this moment, 10 µl of the carotenoid extract dissolved in tetrahydrofuran (THF, Sigma) was added to each well. The serial dilutions of the carotenoid extract were performed taking into consideration only the *all-trans* lycopene content of the extract, and ignoring the other carotenoids, so the final concentration in cell culture medium was between 0.5 µM and 200 µM *all-trans* lycopene. These serial dilutions were used in order to establish the inhibitory concentration 50 (IC50) of the carotenoid extract using the MTT assay.

The cytotoxic effect of the tomato extract was determined by colorimetry using the MTT dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Sigma Aldrich, St Louis, MO, USA). Viable cells have the abilty to transform MTT into an insoluble compound (formazan) due to mitochondrial enzymatic activity. Formazan crystals were solubilized in dimethylsulphoxyde (DMSO, Titolchimica, Italy), followed by photocolorimetric measurement at 570nm, with a Synergy 2.0 microplate reader (BioTek, Winooski, USA). All analysis were performed in triplicate. IC50 values were obtained using a sigmoidal dose-response curve, with a 95% confidence. THF treated cells were used as negative control.

Data was analyzed using the Graph Pad Prism 5 biostatistics software (from GraphPad Software, La Jolla, USA).

RESULTS AND DISCUSSION

The extraction method yielded a satisfactory amount of carotenoids, and proved to be very suitable for this purpose. Also, HPLC-PDA analysis allowed us to easily and precisely characterize the extract, as expected.

The total carotenoid content of tomatoes was 12.31 mg/100 g. The best represented compound was *all-trans* lycopene (6.84 mg/100 g). The other carotenoids were in smaller amounts, as follows: β-carotene 2.96 mg/100 g, γ-carotene 1.44 mg/100 g, lycopene epoxide (0.34 mg/100 g) and *cis*-lycopene (0.73 mg/100 g) (Tab. 1, Fig. 1).

The MTT assay yielded significant results when the tomato extract was added in the culture medium as compared to the negative control (THF treated cells). All analysis were performed in triplicate and the IC50 values found for each determination were comparable, without statistically significant differences between them. The median IC50 value was 84,98 µM *all-trans* lycopene, while the standard error of the mean (SEM) for the three determinations was 3.57

Tab. 1. Carotenoid composition of the tomato extract by HPLC-PDA (peaks are shown in Fig.1)

Peak no.	Compound	Retention time (min)	Amount (mg/100 g)
1	β-Carotene	28.4	2.96
2	γ-Carotene	39.3	1.44
3	Lycopene epoxide	44.3	0.34
4	*All-trans* Lycopene	48.8	6.84
5	*Cis*-Lycopene	49.3	0.73

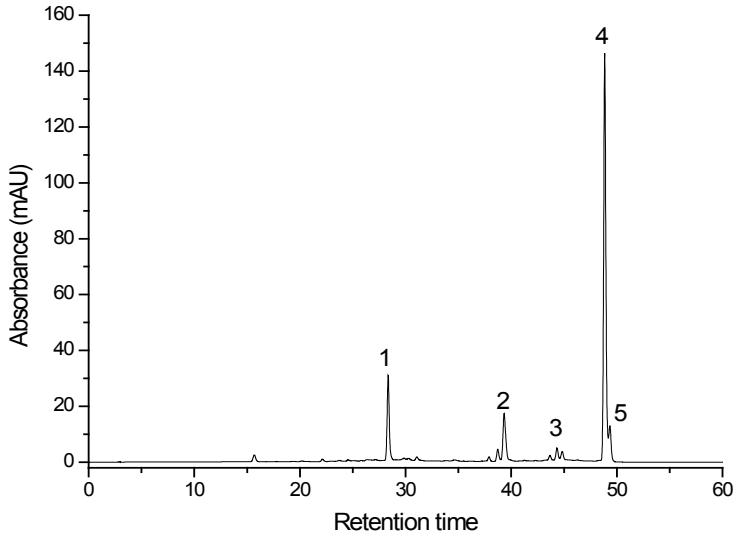

Fig. 1. Chromatogram obtained after HPLC-PDA separation of carotenoids from the tomato extract

(Fig.2). On the other hand, when compared to the negative control, the differences were very significant, demonstrating an obvious negative impact of the carotenoid extract on the HepG2 tumoral cell line (unpaired t-test, one tailed p value <0.05).

Further studies are needed to demonstrate the antioxidant potential of the tomato carotenoids on the hepatocellular carcinoma cell line (HepG2) as well as to prove the capability of these antioxidants to induce apoptosis and inhibit the development of tumoral cells. Another important issue would be to demonstrate the molecular pathways modulated by tomato carotenoids, in order to fully understand their cytotoxic effect.

The work of other authors has shown the antioxidant and cytotoxic potential of lycopene on various tumours. Ilic (2014) reviewed the role of lycopene in inhibiting prostate cancer, revealing a statistically significant correlation between tomato consumption and the decrease of cancer risk. Bilecová-Rabajdová *et al.* (2013) also pointed out the role of lycopene as a chemo-protective agent against the neoplasic disease.

Lung cancer development was also shown to be inhibited by tomato lycopene (Palozza *et al*., 2011) as well as colorectal adenocarcinoma (Slattery *et al.*, 2012). Moreover, breast cancer (Gloria *et al.*, 2014) as well as pituitary adenoma (Haddad *et al.*, 2013) were negatively influenced by tomato-extracted lycopene.

To our knowledge, this is the first study that shows the influence of tomato-extracted carotenoids on hepatocellular carcinoma cells, namely the HepG2 cell line.

One of the most commonly observed pathway that is modulated by lycopene in tumoral cells is NF-κB. At the molecular level, most types of human tumors isolated from patients had abnormalities in the NF-κB signaling pathway, which is constitutively active (Jing and Lee, 2014). Therefore, the genes responsible for cell

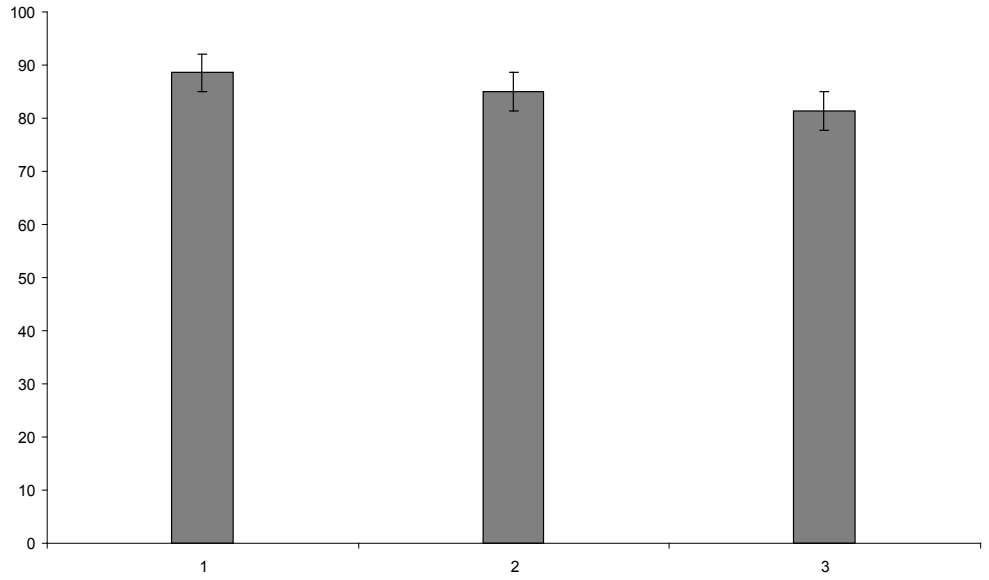

Fig. 2 Graphic representation of cell viability, showing the IC50 concentrations obtained after performing the MTT assay

proliferation become active, as well as preventing phenomena that would normally lead to cell death by apoptosis (Ledoux and Perkins, 2014).

Blocking the NF-κB pathway stops cell proliferation, induces apoptosis and makes the cancer cell more susceptible to antitumor agents. Preliminary studies have demonstrated that β-carotene as well has the ability to modulate NF-κB signaling pathway by means of a redox mechanism to tumor cells (Palozza *et al.*, 2003).

CONCLUSION

The extraction method used in our study proved to be a very efficient one, yielding significant amounts of carotenoid pigments, including lycopene, β-carotene, γ-carotene and others, as shown by the HPLC analysis. The antioxidants contained by the extracts showed significant cytotoxicity on HepG2 liver hepatocellular carcinoma cell line, being able to significantly inhibit cellular development in vitro. Therefore, our study showed the cytotoxic influence that antioxidants extracted from tomatoes exert on the in vitro development of a hepatocellular carcinoma cell line, opening new perspectives for other in vitro as well as in vivo studies, involving patients with hepatic cancer.

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