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The antiproliferative effect of carotene juice on lung cancer cells in humans

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Abstract:

Nutrients that exhibit antioxidant properties and are found in plants may be protective against various types of cancers. carotenes present in carrots, which consist of a conjugted system, it is one of the molecules causing these effects. The amis of this work are identify the characteristics of carrote extract and evaluate its anti-cancer cell proliferation activity in vitro assay. carrote extract was obtained by blended of carrote, the carrote extract identified by UV–VIS Spectroscopy , and FTIR technique. Antiproliferative activity was estimated toward lung cancer cell line (HBE140-Human Bronchial Epithelial Cell Lines) by MTT assay. caroten juice have been showed Antiproliferactive effects in lung cancer cell line (HBE140-Human Bronchial Epithelial Cell Lines) in a dose dependent minner (0.2-0.8 µM) with IC50=2.19 µM. Therefore it can be concluded that the carrote extract is a good natural resource as anti-cancer toward HBE140-Human Bronchial Epithelial Cell Lines.

Keywords: caroten, lung cancer, cell line

Introduction

Cancer is considered a non-communicable disease, and is resposible for many deaths around the world [1]. The most common cancer treatments are surgery, chemotherapy, and radiation therapy [2]. Reports indicatethat all these available therapeutic methods did not completely overcome cancer, in addition to their toxic effect on normal tissues [3]. Based on the reasons mentioned, it has become necessary to search for selective and safe treatments. Today, scientific research is directed towards exploring chemical agents extracted from natural plants components that slow or inhibite the development of cell of cancer. Carotenoide compounds is colored pigments in carrot plant, Numerous studies have demonstrated that the carotenoide contribute as antioxidants and anticancer. The literature indicates that carotenoide compounds possess anti-cancer properties [4], as they inhibit a prostate cancer cell line[5], as well as neuroblastoma tumors[6]. Carotene is a precursor to vitamin A , it is considered an essential nutrient that possesses antioxidant activity [7], So, its considered factor of reducing incidence of cancers. It has been proven that cancer risks it is inversely related with elevated in blood carotene or rotinol levels, note that consuming high doses of carotene for long periode of time doses not cause any toxicity, but on the contrary, it is considered a protective factor agasinst cancer [8]. In vitro studies conform carotene is a chemotherapy or chemopreventive treatment against some type of cancer, like skin [9]and prostate cancer [10]. Thus, the goal of current study was evaluate the effect of antiproliferative of carotene in experimental lung cancer.

Material and methods

Extractin: carrots plant was purchsed at a local in Mahaweel, north of the city of babylon. The fresh carrots were washed several times with tape water, then washed with distilled water and left to dry for two hours. After that, the carrot juice was extracted by grinding carrot leaves with 100 ml of distilled water in a blender for 30 minutes. And same steps were performed with carrot pieces to extract carrot juice. After step of extraction, the bio mass was separated from the aqueous solution by centerifuge for 30 min at 5000 rpm (by utilizing a centrifuge, centerifuge, nussloch, Germany). Subsequently, carrot juice was characterized using UV–VIS Spectroscopy by using (UV/VIS-JNWAY, 1600, German) and FTIR, a perkin Elmer Tensor 27 FTIR (Germany).

Cell cultures: the human lung cancer cell line were utilized in vitro study are: 16 HBE140-Human Bronchial Epithelial Cell Lines, A humidified atmosphere was used maintain all cell lines (5% CO_2 and 37 ^{o}C) in medium of DMEM containing 10% fetal bovine serum RPMI (BxPC-3).

Assays of cell Viability: Viability of each cancer cell line was determined by using MTT assay. And after 24 h of incubation cell line with with extracted caroten juice, media of culture was exchanged with fresh media containing MTT (1 mg/ml). And after 2h of incubation, the resulting complex was dissolved in DMSO. Finally, Absorbance was measured at 620 nm utilizing a sunrise ELISA reader.

Statistical analysis

Statistical analysis was achieved by Graphpad prism 8. And the Variations between groups were analyzed by Two-way ANOVA. The statistficance level was set to $P<0.001$.

Results and discussion

Characteristics of carrot extract

UV-VIS spectrum of the carrot juice is show in Figure 1, from the spectrum the cojucated system of carotenoide stracture make its absorbed in the visible area and thus be colored yellowish-orange [11]. Figure 2 shows FTIR spectrum of sample carrot juice.Table 1 demonstrated peaks position of FTIR of sample carrot juice and their interpretations.

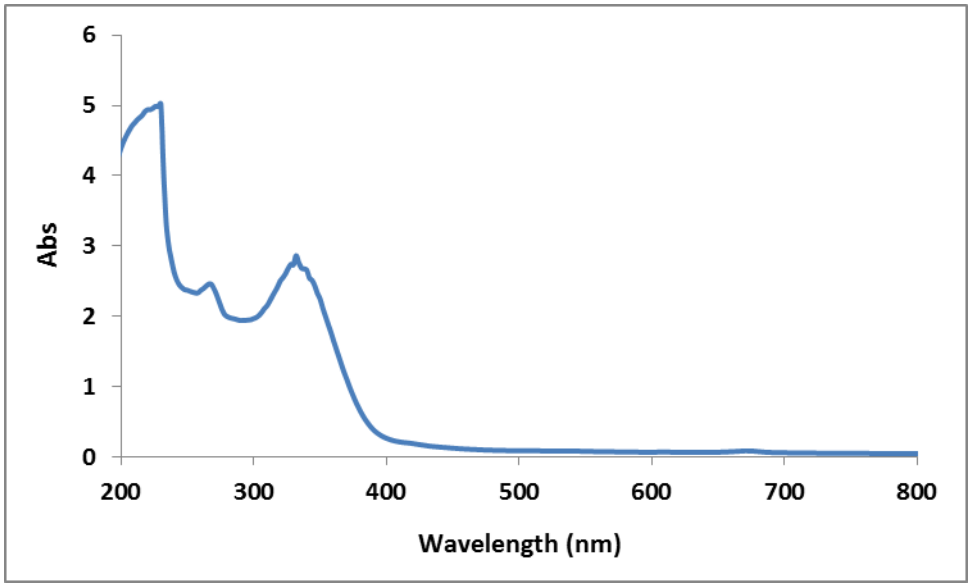

Figure 1. UV-Vis spectrum of sample carrot juice.

Figure 1. shows the FTIR spectrum of carrot juice rich in carotenoids where the bands visble in spectrum are noted in the table 1.

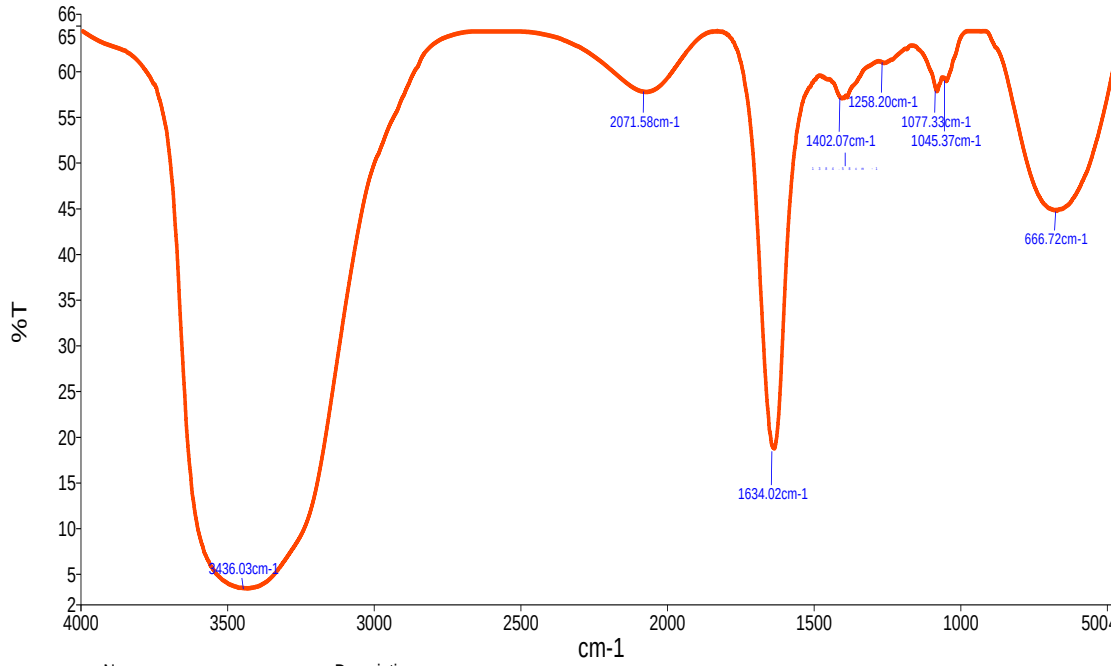

Figure 2. FTIR spectrum of sample carrot juice.

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Table 1. Peaks position of FTIR of sample carrot juice and their interpretations.

No	Beak position (cm^{-1})	Reference (cm^{-1})	Function group
1	3436.03	3200-3450	O-H Stretching
2	2071.58	2100-2350	C=C stretching
3	1634.02	1650-1550	C=C stretching of polyene
4	1402.07	1350-1470	C-H (bending) alphatic
6	1258.20	1000-1300	C-O Streaching

The carrot juice shows absorption at wave number 3436.03 cm^{-1} which attributed to the OH group [12]. in addition, the spectrum indicates the presence of absorption at wave number 2071.58 cm^{-1} which belongs to (C=C) [13]. The peak at 1634.02 cm^{-1} attributed to the absorption of polyene caroteniods, as the literature indicates that absorption appears between 1650-1550 cm^{-1} [14]. There are also an absorptioin at 1402.07 cm^{-1} which indicates the presence of C-H alphatic [15].

Effect of antiproliferactive of carrot juice on human lung cancer cell

Carrot juice effect on lung cancer cell viability, shown decreased viability of cell in dose dependent manner concentration range (0.2-0.8 µM) (table 2). And the lung cancer cell line most sensitive to antiproliferactive effect of concentration of carotien juce was 0.2 µM.

Table 2. Effect of antiproliferactive of caro juice on human lung cancer cell

No	Concentration (µM)	Cell Viability
1	0.8	23.13%
2	0.4	24.7%
3	0.2	78.39%

Recently, many reports have conformed postive impacts of natural pigments on health of human [16]. where carotene is known to have anti-cancer and antimutagenic properties due to its capability to accumlate in tissues of tumor as well as creating a photodynamic effect under laser radiation, and creating reactive oxygen species (ROS) and resulting death of cancer cell [17]. also, the beta-carotene inhibits the growth of many human colon adenocarcinoma cell lines leading to cell cycle arrest and apoptosis [18].

Determination of cytotoxicity by MTT assay

In this work, one type of human lung cancer cell line was used, in the cell proliferation study based on mutation status. The cell line HBE140-Human Bronchial Epithelial Cell Lines was used because of the expression of tyrosine kinase which plays a major role in lung cancer especially in non-small cell lung cancer [19]. Currently, lung cancer is the most dangerouse in the world, as reports indicate that there are more than 1.8 million death because of lung cancerdue to high resistance to current therapy. Therefore, it is necessary to invent new compounds that significantly prevent the development of cancer. In this study we present the anticancer activity of carrot juice extract, as shown in the table 2, the MTT assay showed that the extract has a very high inhibitory activity toward HBE140-Human Bronchial Epithelial Cell Lines with the IC50= 2.19 µg/ml as show in Figure 3, this is consistent with Muammar [20] et al results, where the IC50 for H1975 lung cancer cell treated with the carotene extract was 2.77 µg/ml. From the above results, raw carrot extract can be considered to have anti-cancer avtivity in vitro based on NCI if the IC50 is less than 20 µg/ml [20].

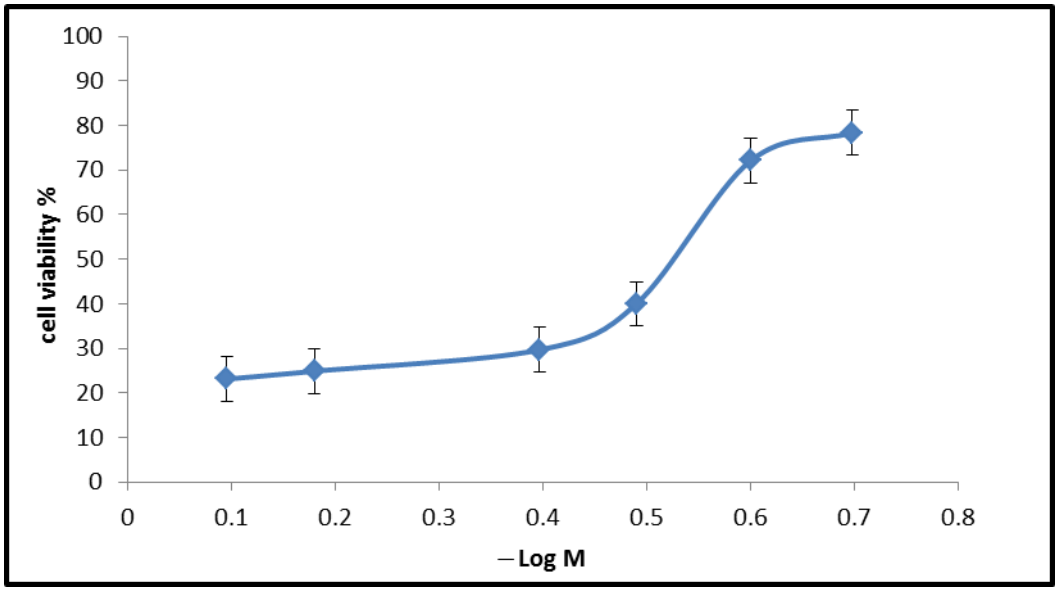

Figure 3. cell viability was expressed by IC50 after exposure to carrot extract by MTT assay.

Conclusions

The cojucated system of carotenoide stracture make its absorbed in the visible area based on the UV-VIS spectroscopy, and this conjucated system are appeared clearly by FTIR spectroscopy. caroten juice has been shown to produce antiproliferactive effects in human lung cancer cell line (HBE140-Human Bronchial Epithelial Cell Lines) by using MTT assay.

Contribution statement of authors

The author confirm that he was the one who completed the practical and writing parts.

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