**Brief Report**
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# Effect of Lutein-Rich Extract on Human Cancer Cells

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## Abstract

**Background:** Lutein and its isomerzeaxanthin are safe natural compounds. They are able to reduce the development of tumor and other chronic diseases. The objectives of the current study was to examine the cytotoxicity of lutein isolated and purified from alfalfa, safe and low-cost plant, on five different human cancer cell lines, namely (MCF-7), (HepG2), (A549), (PC3), and (HCT116), as well as normal (HFB4) cells.

**Method:** We examined the cytotoxicity of lutein purified from Medicago sativa L and evaluate its activity against human liver HepG2, breast MCF-7, lung A549, prostate PC3, and colon HCT116 cancer cell lines using SRB assay in comparison with doxorubicin as a reference drug.

**Results:** Results revealed that the tested extract could be a more promising anticancer agent in the case of MCF-7 (IC50, 3.10±0.47 µg/ml) compared with standard drug doxorubicin (IC50, 2.90±0.30 µg/ml). Moreover, the extract showed a moderate effect on HepG2 (IC50, 6.11±0.84 µg/ml) versus doxorubicin (IC50, 2.90±0.30 µg/ml); meanwhile, the extract showed no activity against A549, PC3, and HCT116 cells. The results further revealed that the extract had no toxicity against the growth of normal HFB4 cells versus doxorubicin.

**Conclusion:** Lutein-rich extract from alfalfa had a major antiproliferative role in breast MCF-7 and liver HepG2 compared to doxorubicin.

***Keywords:*** Carotenoids, Medicago sativa L., Alfalfa, Cytotoxicity, Anticancer

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## Introduction

Medicagosativa L. (alfalfa) is a rich source of vitamins, proteins, minerals, amino acids, and carotenoids. It is a dietary animal food rich in antioxidantxanthophylls,[1] because alfalfa meal contains 400-500 mg carotenoids/kg mainly xanthophylls (lutein and zeaxanthin).[2,3] Our study focused on

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alfalfa as a natural, economic, and safe source of lutein.

There are myriad cancer remedies whose associated restrictions and side-effects have led to the development of other selective, safer, and stronger anticancer agents. The current study tested the cytotoxicity of lutein isolated and purified from alfalfa, safe and low-cost plant, on five different human cancer cell lines, namely MCF-7, HepG2, A549, PC3, and HCT116, as well as normal HFB4 cells.

## Materials and Methods

### *Chemicals*

We obtained the fetal bovine serum (FBS) and L-glutamine from Gibco Invitrogen Company (Scotland, UK). Cambrex (New Jersey, USA) provided Dulbecco's modified Eagle's (DMEM) medium. We purchased dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin, and Sulfo-Rhodamine-B stain (SRB) (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents in this study were of analytical grade and bought from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

### *Cells and culture conditions*

We evaluated the anticancer activity screening of the tested extract against HepG2, MCF-7, A549, PC3, and HCT116 cancer cell lines, as well as, the normal cell line (human normal melanocyte, HFB4). The American Type Culture Collection (Rockville, MD, USA) provided the cells. We maintained the cells in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37oC in a humidified atmosphere containing 5% CO2. Cells at a concentration of $0.50\times10^6$ were grown in a 25 $cm^2$ flask in 5 mL of culture medium.

### *Preparation of lutein from alfalfa*

Ministry of Agriculture, Egypt, identified and supplied fresh alfalfa. After removing its roots, we washed the aerial parts of alfalfa (2.5 K). We ground fresh plant material to extract its juice. Afterwards, by filtration, we separated the juice obtained from plant debris and adjusted the pH to 7-8 with 40% KOH/water. We heated the juice to 93.3°C to coagulate chloroplast into a green curd. After cooling, we separated the curd (containing lutein) from the brown liquor via vacuum filtration; we then dried and extracted the curd using ethyl acetate. The ethyl acetate extract was separated from the solids by vacuum filtration, and totally evaporated using vacuum to obtain crude oleoresin. The oleoresin was saponified by adding 40% KOH/water at (2:1) ratio; the mixture was then strongly shaken at 60°C for 1h using ultrasonic bath. We adjusted the pH of the mixture to 12.We mixed the acetone with the saponified resin at (3:1) (Vol/Vol) ratio at 37°C. After mixing, we separated the acetone solution from the solid state by vacuum filtration. Acetone was evaporated using vacuum, yielding oil rich in lutein.

Finally, we extracted the obtained oil using n-hexane(10:1) (Vol/Vol)at 0°C; lutein crystals were produced with some impurities. The n-hexane was dispelled through filtration. Lutein crystals (70 mg) were washed with water to get rid of impurities and then dried.[4]

### *In vitro antiproliferative activity assay*

We measured the antiproliferative activity of the tested lutein in vitro using the Sulfo-Rhodamine-B stain (SRB) assay according to Skehanet al. (1990).[5] Briefly, cells were inoculated in a 96-well microtiter plate (104 cells/ well) for 24 h prior to treatment with the tested lutein to allow the cells to attach to the wall of the plate. The tested lutein was dissolved in DMSO at 1 mg/mL immediately before use and diluted to the appropriate volume just prior to addition to the cell culture. We added different concentrations of the tested lutein extract and doxorubicin to the cells. We prepared three wells for each individual dose. Monolayer cells were incubated with the extract for 48 h at 37°C under 5% $CO_2$. After 48 h, the cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1%

**Table 1.** In vitro cytotoxic activity of the tested extract expressed as $IC_{50}$ values on different cell lines

| | $IC_{50}$ (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | MCF-7 | HepG2 | A549 | HCT116 | PC3 | HFB4 |
| Doxorubicin | 2.90±0.30 | 3.80±0.37 | 4.28±0.52 | 5.24±0.47 | 5.33±0.61 | 87.65±10.11 |
| Extract | 3.10±0.47 | 6.11±0.84 | 26.77±3.49 | 37.11±5.82 | 56.19±7.11 | 81.60±9.82 |

Data were expressed as mean±SD of three independent experiments

acetic acid. We removed the unbound dye by four washes with 1% acetic acid and recovered the attached stain with Tris-EDTA buffer. An ELISA reader measured the color intensity. We plotted the relationship between surviving fraction and drug concentration to obtain the survival curve for each cell line following the specified time. Table 1 shows the calculated concentrations required for 50% inhibition of cell viability ($IC_{50}$). We compared the result with the antiproliferative effects of the reference control doxorubicin.[6]

### *Statistical analysis*

Data were reported as mean±S.E. (Standard Error) of three independent experiments.

## Results

### *Antiproliferative activity of the extract*

As shown in table 1, we assessed the antiproliferative activity of the tested lutein extract against human MCF-7, HepG2, A549, PC3, and HCT116 cancer cell lines using SRB assay and compared the extract with doxorubicin as a reference drug. The results revealed that the tested extract did not exert any activity against human A549, PC3, and HCT116 cells. The extract showed significant anticancer activity against MCF-7 and HepG2 cells. We observed the extract to be a potent anticancer agent with an $IC_{50}$ value of 3.10±0.47 µg/mlL, which is close to that of the standard drug doxorubicin in MCF-7 cancer cells ($IC_{50}$ 2.90±0.30 µg/mL). Moreover, the extract revealed a moderate effect on HepG2 cells with $IC_{50}$ value 6.11±0.84 µg/mL versus 2.90±0.30 µg/mL for doxorubicin. It is clear that the examined lutein extract was more effective against breast cancer in comparison with hepatic cells. Additionally, the extract had no toxicity against the growth of normal HFB4 cells as compared to the doxorubicin.

## Discussion

In the current study, we examined the antiproliferative effect of lutein extracted and purified from alfalfa on five human cancer cell lines, namely MCF-7, HepG2, A549, PC3, and HCT116, as well as, normal HFB4 cells. Our results showed that lutein had a significant antitumor effect on human cancer cell lines. Lutein not only conjugated double bonds responsible for the free radical scavenging system, but also had two terminal hydroxyl groups on both ends, making it a strong antioxidant compared to other carotenoids.[7] In addition, lutein was more active than β-carotene in suppressing the cell lipids autoxidation[8] and preventing oxidant-induced celldamage.[9]

The findings of the current study showed that the lutein had a potent antitumor activity similar to doxorubicin in MCF-7 cancer cell line. Rocket al. (1996) reported that the high content of dietary lutein in humans was related to the high expression of estrogen receptors in breast tumor cells, hence the high survival rates and perfect response to hormone therapy.[10] Our findings revealed that the tested lutein-rich extract had a moderate activity compared to doxorubicin in HepG2 cell line. Moreno et al. (2007)[11] revealed that lutein was able to inhibit, but not block, activities through the initial state of hepatocarcinogenesis, thereby playing a vital role as a suppressing agent. The tested extract had no cytotoxicity on A549, PC3, and HCT116 cancer cell lines. Nishino et al.(2009) investigated the effect of lutein on the lung carcinogenesis of ddY mice and reported that lutein had an antitumor activity against lung carcinogenesis.[12] There is little information concerning the antitumor activity of lutein against colon and prostate carcinogenesis. However, Gunasekera et al. (2007) reported that lycopene, lutein, or their combination inhibited the growth

of prostate tumor cell (AT3).[13] Furthermore, test extract had no cytotoxicity against the normal cell line (human normal melanocyte, HFB4) compared to doxorubicin, which is in agreement with FAD. Therefore, lutein is generally regarded as a safe (GRAS) and non-toxic phytochemical agent.[6]

## Conclusion

We conclude that lutein-rich extract from alfalfa had a major antiproliferative role in breast MCF-7 and liver HepG2 compared to doxorubicin.

## Conflicts of Interest

None declared.

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