Effect of Lutein-Rich Extract on Human Cancer Cells

Wesam Mostafa Omar*, BSc, Amr E. Ahmed*, PhD, Mai Raslan*, PhD, Khalid El-Nesr**, PhD, Mamdouh Moawad Ali***, PhD, Mohamed De Abdelmaksoud***, PhD, Dina El Dahshan****

*Biotechnology and Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt
**Pathology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt
***Biochemistry Department, National Research Centre, Cairo, Egypt
****Clinical Pathology Department, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

Abstract

Background: Lutein and its isomer zeaxanthin 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

Introduction

Medicago sativa L. (alfalfa) is a rich source of vitamins, proteins, minerals, amino acids, and carotenoids. It is a dietary animal food rich in antioxidant xanthophylls, because alfalfa meal contains 400-500 mg carotenoids/kg mainly xanthophylls (lutein and zeaxanthin). Our study focused on
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 37°C in a humidified atmosphere containing 5% CO2. Cells at a concentration of 0.50×10^6 were grown in a 25 cm² 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.

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 Skehan et al. (1990). 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% CO2. After 48 h, the cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1%
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).

<table>
<thead>
<tr>
<th>IC50 (µg/mL)</th>
<th>MCF-7</th>
<th>HepG2</th>
<th>A549</th>
<th>HCT116</th>
<th>PC3</th>
<th>HFB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>2.90±0.30</td>
<td>3.80±0.37</td>
<td>4.28±0.52</td>
<td>5.24±0.47</td>
<td>5.33±0.61</td>
<td>87.65±10.11</td>
</tr>
<tr>
<td>Extract</td>
<td>3.10±0.47</td>
<td>6.11±0.84</td>
<td>26.77±3.49</td>
<td>37.11±5.82</td>
<td>56.19±7.11</td>
<td>81.60±9.82</td>
</tr>
</tbody>
</table>

Data were expressed as mean±SD of three independent experiments.

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 antipro-
liferative 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 IC50 value of 3.10±0.47
µg/mL, which is close to that of the standard drug
doxorubicin in MCF-7 cancer cells (IC50 2.90±0.30
µg/mL). Moreover, the extract revealed a moderate
effect on HepG2 cells with IC50 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. In addition, lutein
was more active than β-carotene in suppressing
the cell lipids autoxidation and preventing
oxidant-induced cell damage.

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
et 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. Our findings revealed that
the tested lutein-rich extract had a moderate
activity compared to doxorubicin in HepG2 cell
line. Moreno et al. (2007) 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. 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

Table 1. In vitro cytotoxic activity of the tested extract expressed as IC50 values on different cell lines

<table>
<thead>
<tr>
<th></th>
<th>MCF-7</th>
<th>HepG2</th>
<th>A549</th>
<th>HCT116</th>
<th>PC3</th>
<th>HFB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>2.90±0.30</td>
<td>3.80±0.37</td>
<td>4.28±0.52</td>
<td>5.24±0.47</td>
<td>5.33±0.61</td>
<td>87.65±10.11</td>
</tr>
<tr>
<td>Extract</td>
<td>3.10±0.47</td>
<td>6.11±0.84</td>
<td>26.77±3.49</td>
<td>37.11±5.82</td>
<td>56.19±7.11</td>
<td>81.60±9.82</td>
</tr>
</tbody>
</table>
of prostate tumor cell (AT3). 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.

### 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.

### References