Studies of Cytotoxic Potential of Cucumis melo. Linn Fruit Aqueous Extract in Prostate Cancer Cell lines PC-3 Using MTT and Neutral Red Assay

SIBI P ITTIYAVIRAH, ANN GEORGE, ANJU M SANTHOSH, SUDHI T KURIAN, PRINSY PAPPACHAN and GIFTY JACOB

ABSTRACT

The objective of the study was to evaluate the cytotoxic effects of aqueous fruit extract of Cucumis melo in human prostate cancer cell line (PC-3) using MTT and neutral red assays. The crude aqueous extract of C. melo was prepared by cold maceration method, filtered, concentrated and tested on PC-3 cell line. Dose-dependent cytotoxic activities were exhibited by human prostate carcinoma PC-3 cell line. As the dose of the extract increased, the number of viable cells decreased. This confirms the anti-cancer and cytotoxic potential of the fruit of C. melo.

Keywords: Cytotoxicity, MTT assay, Neutral red assay, Human prostate carcinoma cell lines (PC-3), Cucumis melo
antioxidant molecule. Most of these plant extracts were mixed with strained liquid, filtered to make a clear extract. The following tests were carried out to analyze the possible phytochemicals present in the aqueous extract of C. melo.

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MATERIALS AND METHODS

Plant material

C. melo fruits were collected from local fruit stall in Cherthala, Alappuzha District in the month of November 2012 and authenticated at Department of Environment Sciences, Mahatma Gandhi University, Kottayam, Kerala, India. In vitro methods were used for assessing the cytotoxic activity and they were in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC).

Reagents for phyto-chemical analysis

Bismuth nitrate, Nitric acid, Potassium iodide, Sodium carbonate, Mercuric chloride, Sulphuric acid, Hydrochloric acid, Sodium hydroxide, Ferric chloride, Alpha naphthol, Copper sulphate, Zinc chloride 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide(MTT), Isopropanol, Phosphate buffer saline(PBS), Dimethyl sulfoxide (DMSO), Calorimeter, Flavonoids

Phytosterols

1M Potassium dihydrogen phosphate, CO₂ Incubator, PBS, Elution medium (ethanol/acetate acid), Spectrophotometer.

MTT assay and neutral red assay

PC-3 cell line-PC-3 prostate cell lines purchased from National centre for Cell Sciences (NCCS), Pune, Maharashtra, India. 1M Potassium dihydrogen phosphate, CO₂ Incubator, PBS, Elution medium, (ethanol/acetate acid).

Preparation of extract: cold maceration

The fruit was washed and the outer skin was peeled off. The remaining fleshy part was cut in to small pieces. Then it was soaked in water for seven days. A small amount of alcoholic extract was dissolved in 1 ml of water and the aqueous NaOH solution was done occasionally. After seven days, the liquid was added. Formation of yellow colour indicated the presence of flavonoids.

Glycosides

1M Potassium dihydrogen phosphate, CO₂ Incubator, PBS, Elution medium, (ethanol/acetate acid).

Preparation of extract: cold maceration

The fruit was washed and the outer skin was peeled off. The remaining fleshy part was cut in to small pieces. Then it was soaked in water for seven days. A small amount of alcoholic extract was dissolved in 1 ml of water and the aqueous NaOH solution was done occasionally. After seven days, the liquid was added. Formation of yellow colour indicated the presence of flavonoids.
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Abs 0.2 was removed with 10.148 M
Inference Absence of carboh
nucubated at room temper

(µg, 500 × PBS. Then,
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<th>Table 2. Cytotoxicity studies aqueous extract of C. melo using MTT assay</th>
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<tr>
<td>Sample concentration (µg/ml)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>500</td>
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<td>1000</td>
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</table>

Tannins-Ferric chloride test
To 1-2 ml of aqueous extract, few drops of 5%
aqueous ferric chloride solution was added. A bluish
black color which disappears on addition of a few ml of
sulphuric acid. There is no formation of yellowish brown
precipitate.

Sugars-Molisch’s test
In a test tube containing 2 ml of aqueous extract, 2-
drops of freshly-prepared 20% alcoholic solution of α-
naphthol was added and mixed. To this solution, 2 ml of
con: Sulfuric acid was added so as to form a layer
below the mixture. Formation of red violet ring at the
junction of solution and its disappearance on the
addition of an excess solution indicated the presence of
carbohydrates.

Proteins-Biurett’s test
In a test tube containing 2 ml of test sample, 2 ml of
10% NaOH is added and mixed well. Then 0.1% CuSO4
solution is added. A violet or pink colour indicated the
presence of proteins.

Saponins-Froth Test
Few ml of the extract is transferred in to a test tube
and shaken vigorously then is left to stand for 10 min. A
thick persistent froth indicated presence of saponins.

MTT assay
MTT is a colorimetric assay that measures the
reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-
diphenyl tetrazolium bromide (MTT) by mitochondrial
succinate dehydrogenase. The MTT enters the cells and
passes into the mitochondria where it is reduced to an
insoluble, colored (dark purple) formazan product. The
products are then solubilized with an organic solvent
(eg. isopropanol) and the released, solubilized formazan
reagent. Since reduction of MTT can only occur in
metabolically-active cells, the level of activity is a
measure of the viability of the cells

PC-3 human prostate cell lines purchased from
NCCS Pune, was maintained in Dulbecco’s Modified
Eagles Media (DMEM) and grown to confluency at
37°C and 5% CO2 in a humidified atmosphere in a CO2
incubator. The cells were trypsinized (500 µl of 0.025%
Trypsin in PBS/ EDTA solution) for 2 min and
passaged to T flasks in complete aseptic conditions and
incubated. Extracts were added to 80% confluent cells
at a concentration of 100 µg, 500 µg and 1000 µg from a
stock of 100 mg/ml and incubated for 24 h. The cell
culture suspension was washed with 1× PBS. Then, 200
µl MTT solutions were added to the culture (MTT: 5
mg/volume dissolved in PBS). These were incubated at
37°C for 3 hours. All MTT wash was removed with 1×
PBS and 300 µl DMSO was added to each culture. The
plates were incubated at room temperature for 30 min
until the cell get lysed and color was obtained. The
solution was transferred to centrifuge tubes and
centrifuged at top speed for 2 min to precipitate cell
debris. Optical density (OD) was read at 540 nm using
DMSO as blank.

Neutral red assay
The neutral red cytotoxicity test was based on the
ability of living cells to uptake and bind neutral red
(NR). NR was a positively-charged dye that easily
diffuses through the cellular membrane of the cells,
accumulates in the cellular cytoplasm and stores in the
acidic environment of lysosomes. The principle of the
test consists in the fact that NR are able to absorb and
bind only with live cells while this ability declines in
damaged or dead cells. The amount of accumulated NR
was thus directly proportional to the amount of live cells
in the cell culture. The pH of the neutral red solution
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**RESULTS**

**Phytochemical analysis**

The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [8]. The Phytochemical screening of the aqueous extracts of plant sample revealed the presence of alkaloids and flavonoids and phytosterols (Table 1) [9].

**Cytotoxic studies**

MTT results showed that 1000 µg/ml aqueous extract of *Cucumis melo* showed 67.27% (Table 2, Fig 1) while the neutral red uptake assay showed 66.27% (Fig 2) viability (Table 3, Fig 2). The photograph of PC-3 cell line is taken using inverted illuminating microscope while the control received vehicle and treatment groups were received *C. melo* at a conc.1000 µg/ml and 1000 µg/ml (Fig 3). The results indicate that the crude aqueous extract of *C. melo* on PC-3 cell lines had shown a dose-dependent anti proliferative effect. The IC₅₀ values of MTT and Neutral red assays were found to be 1470 and 1860 µg/ml respectively (Figs 4 and 5).

**DISCUSSION**

Metastatic prostate carcinoma is associated with a high morbidity and mortality rate with a medium survival of approximately, 12–15 months. Available treatment alternatives include radiotherapy after radical retropubic prostatectomy, radical prostatectomy, external beam radiation, prostate brachy therapy, and androgen ablation of the prostate. Until recently, despite androgen suppression, no cytotoxic agent has been able to change the progression of metastatic prostate cancer. Androgen ablation therapy remains the main course of treatment with advanced disease. However, it has no effect on hormone-independent cancer cells. Chemotherapeutic agents result in less than a 10% response in advanced prostate carcinoma, in part due to increased resistance of androgen-independent cells to apoptosis. However, the severe side effects of chemotherapy have remained a major problem. In recent years considerable efforts have been made to identify naturally-occurring compounds and related synthetic agents can prevent the development and recurrence of cancer. A wide variety of natural food and food products can induce apoptosis in various tumor cells. There is strong evidence supporting the positive role of some natural materials and medicinal plants in oncology and their ability affect all phases of tumorigenic process. Therefore, it is important to screen the natural products either as crude extracts or as isolated components for apoptotic properties to identify extract of *Cucumis melo* (Table 2, Fig 1) while the neutral red uptake assay showed 66.27% (Fig 2). Agents currently used are derived from natural sources, including plants, marine organisms and micro-

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**Table 3. Cytotoxicity studies aqueous extract of *C. melo* using Neutral red assay**

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<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>OD (540 nm)</th>
<th>% viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.086</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>0.070</td>
<td>81.39</td>
</tr>
<tr>
<td>500</td>
<td>0.065</td>
<td>75.58</td>
</tr>
<tr>
<td>1000</td>
<td>0.057</td>
<td>66.27</td>
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**Fig 1. Cytotoxicity studies aqueous extract of *C. melo* using MTT assay**

**Fig 2. Cytotoxicity studies aqueous extract of *C. melo* using Neutral red assay**

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organisms and they offer an opportunity to study the mechanisms of tumorgenesis. Cucurbitaceae plants are highly useful as they have good potential against many health ailments. In the present study, the phytochemical screening of the aqueous extracts of plant sample revealed the presence of alkaloids and flavonoids and phytoestrols [9]. These phytoconstituents may be responsible for various activities. Flavanoids are diverse family of compounds commonly found in fruits, vegetables and honey. Flavanoids are generally safe and associated with low toxicity, making them ideal candidates for cancer chemopreventive agents. MTT results and neutral red uptake assay confirms dose-dependent anti-proliferative effect of crude aqueous extract of Cucumis melo on prostate cancer cell lines. As the dose of the extract increases, number of viable cell decreases and confirms the cytotoxic activity.

It is concluded that the aqueous extract of C. melo was found to possess dose-dependent cytotoxic activity on metastatic human prostate cancer cell lines PC-3. Further studies are warranted to explore the anticancer effect of C. melo and also the active principles could be isolated and investigated.

**Fig 3.** Photograph of PC-3 cell line: i) control received vehicle, ii) C.melo at a conc. 100µg/ml and iii) C.melo at a conc. 1000 µg/ml

**Fig 4.** IC50 value of aqueous extract of C.melo using MTT assay

**Fig 5.** IC50 value of aqu. extract of C.melo using Neutral red assay


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Anju M Santhosh, Department of Pharmacology, University College of Pharmacy, Cheruvandoor campus, Ettumanoor P.O, Kottayam, Kerala Pin-686691, India.

Sudhi T Kurian, Department of Pharmacology, University College of Pharmacy, Cheruvandoor campus, Ettumanoor P.O, Kottayam, Kerala Pin-686691, India.

Princy Pappachan, Department of Pharmacology, University College of Pharmacy, Cheruvandoor campus, Ettumanoor P.O, Kottayam, Kerala Pin-686691, India.

Gifty Jacob, Department of Pharmacology, University College of Pharmacy, Cheruvandoor campus, Ettumanoor P.O, Kottayam, Kerala Pin-686691, India.

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