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

Carcinoma of the prostate gland is the malignancy of men, with mean age at presentation about 70 years. Treatment of Kidney stones, cancer, cardiovascular when diagnosed by the presence of symptoms. The risk disorders and stroke. Three components found in of developing prostate cancer is affected by racial and melons are Cucubitacin-β, Lithium and Zinc which environmental factors. The magnitude of familial risk exhibit promise in cancer prevention, fighting increases with number of first degree relatives who are depression, dandruff, and ulcers and stimulates the affected, and also if the affected relatives diagnosed immune system. *C.melo* is also rich in antioxidants, with prostate cancer at an early age. Prostate cancer risk flavonoids such as β-carotene, lentin, xanthin and has been inversely associated with several dietary cryptoxanthin. These antioxidants have the ability to components including the essential non-metallic trace protect cells and structures in the body from oxygen free radicals, hence offer protection against prostate, colon, associated with an inherited predisposition to prostate breast, lungs, endometrial and pancreatic cancer. The cancer and prostate cancer and its development. Two cucurbitaceae family includes several species of prostate cancer susceptibility genes that have been cultivated plants that has great economic importance identified are the RNASEL and MSR1 genes both of like water melon (*Citrus lanatus* L.), squash which are associated with response to infections. (*Cucurbita maxima* L.), cucumber (*Cucumis sativus* L).

*Cucumis melo* fruit is round in shape, tan to greenish and cantaloupe(*Cucumis melo*. L.) [1]. Earlier studies on tan with a rough texture and orange pink flesh. It is well known for its sweet taste and fragrance. It is native to Persia, Armenia, etc. Many phytochemicals having properties [2]. The active principles in the vegetable potential benefits are present in *C. melo*. It is rich in extracts are principally water soluble or lipophilic
antioxidant molecule. Most of these plant extracts were mixed with strained liquid, filtered to make a clear solution containing various amounts of vitamin E and C, Carotenes, liquid and concentrated triterpenoids and other flavonoids [3]. For this, these were used as potential antioxidant prophylactic agents for both health and diseases management [3,4]. The methanolic seed extract (MECM) of Cucumis melo.Vars. 1.2, which possess significant antioxidant, anti-inflammatory and analgesic properties [5], while the fruit extract C. melo., fruit exhibited immunomodulatory activity [6]. Even though a large number of compounds were screened for cytoxicity and anticancer studies, hardly a few lead compounds had shown promising results. Hence, it was thought to identify potential compounds from our traditional ethno-medicinal knowledge for treatment of kidney, urinary and prostate cancer. In the present study, an initial attempt has made for to scientifically evaluate its anticancer effects. The main aim of the study is to evaluate the cytoxic effects of aqueous fruit extract of C. melo in human prostate cancer cell line (PC-3) using MTT and neutral red assays.

MATERIALS AND METHODS

Plant material

C. melo fruits were collected from local fruit stall Cherthala, Alappuzha District in the month of November 2012 and authenticated at Department of Environment Sciences, Mahatma Gandi University, Kottayam, Kerala, India. In vitro methods were used for assessing the cytoxic 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-yldiphenyl tetrazolium bromide(MTT), Isopropanol, Phosphate buffer saline(PBS), Dimethyl sulfoxide (DMSO), Calorimet, Flavonoids

1M Potassium dihydrogen phosphate , CO2 incubator, PBS, Elution medium (ethanol/acetic 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/acetic 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 and was kept in a dark place. During this period shaking was done occasionally. After seven days, the liquid was added. Formation of yellow colour indicated the strained and marc was pressed. The expressed liquid presence of glycosides.

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Published online: January 31, 2013
The amount of accumulated NR solution and its disappearance on the addition of a few ml of aqueous extract, 2 ml of PBS. Then, to this solution, 2 ml of freshly-prepared 20% alcoholic solution of anaphthol was added and mixed. To this solution, 2 ml of conc. 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 tannins.

**Biurett's test**

In a test tube containing 2 ml of aqueous extract, 2 ml of 10% NaOH was added and mixed well. Then 0.1% CuSO₄ solution is added. A violet or pink colour indicated the presence of proteins.

**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.

**Neutral red assay**

The neutral red cytotoxicity test was based on the ability of living cells to uptake and bind neutral red (NR). NR is 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 was 7.4.

**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.

Table 1. Phytochemical constituents aqueous extract of *C. melo*

<table>
<thead>
<tr>
<th>Tests</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dragenhoff's</td>
<td>Orange red ppt was observed</td>
<td>Presence of alkaloids (+)</td>
</tr>
<tr>
<td>Mayer's test</td>
<td>Pale white ppt was observed</td>
<td>Presence of alkaloids (+)</td>
</tr>
<tr>
<td><strong>Flavanoids</strong></td>
<td>Dirty brown ppt was observed</td>
<td>Presence of Flavanoids (+)</td>
</tr>
<tr>
<td><strong>Phytosterols</strong></td>
<td>Dark golden colour was observed</td>
<td>Presence of phytosterols (+)</td>
</tr>
<tr>
<td><strong>Glycosides</strong></td>
<td>No characteristic change was observed</td>
<td>Absence of glycosides (-)</td>
</tr>
<tr>
<td><strong>Tannins</strong></td>
<td>No characteristic change was observed</td>
<td>Absence of glycosides (-)</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molisch's test</td>
<td>No characteristic change was observed</td>
<td>Absence of carbohydrates (-)</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>No characteristic change was observed</td>
<td>Absence of proteins (-)</td>
</tr>
<tr>
<td><strong>Saponins</strong></td>
<td>No characteristic change was observed</td>
<td>Absence of saponins (-)</td>
</tr>
</tbody>
</table>

Table 2. Cytotoxicity studies aqueous extract of *C. melo* using MTT assay

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>OD (540 nm)</th>
<th>% viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.220</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>0.211</td>
<td>95.90</td>
</tr>
<tr>
<td>500</td>
<td>0.172</td>
<td>78.18</td>
</tr>
<tr>
<td>1000</td>
<td>0.148</td>
<td>67.27</td>
</tr>
</tbody>
</table>

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.

**Tannins-Ferric chloride test**

In a test tube containing 2 ml of aqueous extract, 2 ml of freshly-prepared 20% alcoholic solution of anaphthol was added and mixed. To this solution, 2 ml of conc. 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.

**Sugars-Molisch’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.

**Cytoxicity studies [7]**

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

<table>
<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>
</tr>
</tbody>
</table>

Recent studies have shown that the crude products either as crude extracts or as isolated components for apoptotic properties to identify potential anti-cancer compounds. Over 60% anti-cancer agents currently used are derived from natural sources, including plants, marine organisms and microorganisms. Cytotoxic studies aqueous extract of Cucumis melo showed 67.27% (Table 2, Fig 1) while the control neutral red assay showed 66.27% 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.100µg/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 brachytherapy, 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 tumorogenic process. Therefore, it is important to screen the natural products either as crude extracts or as isolated components for apoptotic properties to identify potential anti-cancer compounds. Over 60% anti-cancer agents currently used are derived from natural sources, including plants, marine organisms and microorganisms.
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

Organisms and they offer an opportunity to study the molecular 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 phytosterols [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 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 cells 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.

REFERENCES


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Published online: January 31, 2013