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

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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 the male genitourinary tract and is a disorder in older 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 Cucurbitacin-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 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 element selenium. Chromosomal alterations are radicals, hence offer protection against prostate, colon, associated with an inherited predisposition to prostate breast, lungs, endometrial and pancreatic cancer. The gland and prostate cancer and its development. Two *Cucurbitaceae* family includes several species of cultivated plants that has great economic importance *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
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antioxidant molecule. Most of these plant extracts contain various amounts of vitamin E and C, Carotenoids, 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* var. 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 cytotoxicity and anticancer studies, hardly a few lead compounds had shown promising results. Hence, it was thought to identify potential compounds from our traditional ethno-medical knowledge for treatment of kidney, urinary and prostate cancer. In the present study, an initial attempt has made to scientifically evaluate its anticancer effects. The main aim of the study is to evaluate the cytotoxic 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 stalls in 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 cytotoxic activity and they were in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC).

### Reagents for phyto-chemical analysis

- Bismuth nitrate, Nitric acid, Potassium iodide [46]
- The amount of 1.2 g Iodide and 2.0 g of H₂SO₄ and Sodium carbonate, Mercuric chloride, Sulphuric acid [47]
- The solution was diluted to 100 ml. Ten ml of alcoholic Hydrochloric acid, Sodium hydroxide, Ferric chloride [48]
- The extract was identified by adding 1.5% v/v of HCl and a Alpha naphthol, Copper sulphate, Zinc chloride 3-(4,5) [49]
- Few drops of Wagner's reagent. Formation of yellow or dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium [50]
- Brown precipitate confirmed the presence of alkaloid.
- Bromide(MTT), Isopropanol, Phosphate buffer
- salin(PBS), Dimethyl sulfoxide (DMSO), Calorimeter, [51]
- **Flavonoids**
- 1M Potassium dihydrogen phosphate, CO₂ Incubator, PBS, Elution medium (ethanol/acetic acid), [52]
- 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), [53]
- **Phytosterols**
- To 2 ml of chloroform extract, 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. In the presence of phytosterols, a golden yellow color was produced in the chloroform layer.
- **Glycosides**
- 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 color indicated the strain and marc was pressed. The expressed liquid presence of glycosides.

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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>Orange red ppt was observed</td>
<td>Presence of alkaloids (+)</td>
</tr>
<tr>
<td>Drageodroff’s test</td>
<td>Pale white ppt was observed</td>
<td>Presence of alkaloids (+)</td>
</tr>
<tr>
<td>Mayer’s test</td>
<td>Dirty brown ppt was observed</td>
<td>Presence of Flavonoids (+)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Dark golden colour was observed</td>
<td>Presence of phytosterols (+)</td>
</tr>
<tr>
<td>Glycosides</td>
<td>No characteristic change was observed</td>
<td>Absence of glycosides (+)</td>
</tr>
<tr>
<td>Tannins</td>
<td>No characteristic change was observed</td>
<td>Absence of tannins (-)</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>No characteristic change was observed</td>
<td>Absence of carbohydrates (-)</td>
</tr>
<tr>
<td>Molisch’s test</td>
<td>Pale white ppt was observed</td>
<td>Presence of carbohydrates</td>
</tr>
<tr>
<td>Proteins</td>
<td>No characteristic change was observed</td>
<td>Absence of proteins (-)</td>
</tr>
<tr>
<td>Saponins</td>
<td>Pale white ppt was observed</td>
<td>Presence 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>

167 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.

173 Sugars-Molisch’s test

In a test tube containing 2 ml of aqueous extract, 2 ml of freshly-prepared 20% alcoholic solution of α-naphthol 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.

182 Proteins-Biuret’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 color indicated the presence of proteins.

187 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.

193 Cytoxicity studies [7] 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.

204 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 then passaged to T flasks in complete aseptic conditions and incuabated. 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.

226 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...
was adjusted in all the experiments to 6.35 with the addition of potassium dihydrogen phosphate (1M), 10 μL of neutral red solution was added to plates and incubated for 3 h in CO₂ incubator at 37°C. Cells were then washed with phosphate buffer saline (PBS) and fixed with 200 μl of fixing solution. One ml of the elution medium (ethanol/ acetic acid, 50%/1%) was added followed by gentle shaking for 10 min, so that complete dissolution was achieved. Aliquots of the resulting solutions were transferred to cuvettes and the absorbance at 540 nm was recorded using the spectrophotometer.

**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% (Table 3, Fig 2). The photograph of PC-3 cells including plants, marine organisms and microorganisms is shown in 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 tumorogenic 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* showed 67.27% (Table 2, Fig 1) while the neutral red uptake assay showed 66.27% (Table 3, Fig 2).
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. Flavonoids 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.

REFERENCES


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