"Invitro Cytotoxicity and Free Radical Scavenging Activity of aqueous extract of *Cucumis melo*"

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Abstract

Cucumis melo is a species of melon that has been developed into many cultivated varieties. These include smooth skinned varieties such as honey dew, Crenshaw, casaba and different netted cultivars. It is an accessory fruit of a type called an epigenous berry. Melons are a good source of potassium, vitamin A, folate and generally used as laxative. The aqueous extract of Cucumis melo fruit pulp was found to contain hydroxyl radical, super oxide radical, nitric oxide radical, DPPH activity. It possessed Ferric Reducing Antioxidant Power and was found to possess cytotoxic effect against Ehrlich's Ascites Carcinoma cells. Studies on cytotoxicity broadly involve the metabolic alterations of the cells, including the death of cells as a result of toxic effects of the compounds. In case of anticancer drugs, the deaths of cells were noted. The cancer cells used for the present study is Ehrlich's Ascites Carcinoma that are transplantable, poorly differentiated malignant tumor which appeared originally as a spontaneous breast carcinoma in a mouse. It grows in both solid and ascitic forms. The in vitro cytotoxicity of the aqueous extract of Cucumis melo against Ehrlich's Ascites Carcinoma cells were done at different concentrations 10µg, 20µg, 30µg, 40µg, 50µg, 100µg, 200µg, 300µg, 400µg and 500µg for a period of 3 hours treatment. The control and treated cells were checked for viability by Trypan blue viability method. The stained cells and unstained cells were counted using microscope and percentage of cytotoxicity were calculated. The aqueous extract of Cucumis melo showed cytotoxic effect against the Ehrlich's cancer cells in a dose dependent manner. As the concentration of the extract increased the cytotoxicity also increased. Maximum cytotoxicity was observed at the concentration of 500µg of the extract. 50% of the Cytotoxicity was observed at the concentration of 300µg.

Keywords: Cucumis melo antioxidant evaluation in-vitro cytotoxicity Ehrlich's Ascites Carcinoma cells

Introduction

India is a rich source of medicinal plants and a number of plants extracts are used against diseases in various systems of medicine as Ayurveda, Unani, and Siddha. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes, alkaloids, polysaccharides, and so on have received considerable attention in recent years due to diverse pharmacological properties including cytotoxic and cancer chemo preventive effects. Several plant products have been tested for anticancer activity and some of them like vincristine, taxol and so on are now available as drug of choice[8]. The fruit selected for the present study is

Cucumis melo also called as musk melon. The plant is a long trailing annual vine that belongs to cucumber family. It grows in sandy areas and also near river banks. It is a good source for appetite, weight loss, urinary tract infections, constipation, acidity, and ulcers.[2]

Free radical is any atom or a molecule which has a single electron on its external orbit. Unstable free radicals are produced in normal metabolism when oxygen is used to burn food for energy. A free radical is defined as "any species capable of independent existence that contains one or more unpaired electrons". Generally, free radicals are more reactive than non radicals and will react with them to produce new free radicals in a chain reaction[5].Cytotoxicity is the quality of being toxic to cells. A chemical substance, an immune cell, or some types of venom are toxic agents. Treating cells with a cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing, or the cells can activate a genetic program of cell death, apoptosis. Cells undergoing necrosis typically exhibit rapid swelling, lose membrane integrity, shut down metabolism, and release their contents into the environment. Cells that undergo rapid necrosis in vitro do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers. Apoptosis

is characterized by well defined cytological and molecular events including a change in the refractive index of the cell, cytoplasm shrinkage, nuclear condensation, and cleavage of DNA into regularly sized fragments. Cells in culture that are undergoing apoptosis eventually undergo secondary necrosis. They will shut down metabolism, lose membrane integrity and lyse.[3] The Present study focuses on the invitro Cytotoxicity and Free Radical Scavenging Activity of aqueous extract of *cucumis melo.*

Materials and Methods

Collection and Preparation of Sample: The fruit was collected from the local farmers and has been authenticated by the Botanical Survey of India. The pulp of fresh fruits was chopped into pieces and dried at room temperature for 24hours. The air dried pulps were kept at 40ºC in hot air oven for 24hours to remove moisture content. The completely dried fruits were ground into powder by using mixer grinder. 50g of the dried fruit powder was mixed in the ratio (w/v) of 1:10 with water. The extraction was carried out in a shaker water bath at 40ºC for 48 hours and filtered through Whatmann No 1 filter paper. The extract was concentrated to dryness and stored in deep freezer.

Collection of Cell Line: The Ehrlich's Ascites Carcinoma cells were collected from Amala Cancer Institute, Kerala, India.

Free Radical Scavenging Activity

Hydroxyl Radical Scavenging Activity : The reaction mixture contained Deoxyribose (2.8mM); $FeCl_2$(100mM); KH_2PO_4-KOH buffer (20mM,pH 7.4); EDTA(100mM); H_2O_2 (1.0mM); ascorbic acid (100mM), and various concentrations of the extract s in a final volume of 1ml. Ferric chloride and EDTA were premixed just before addition to the reaction mixture. The reaction mixture was incubated at 37ºC for 1hour. 1.0ml of 2.8% Trichloroacetic acid and 1.0ml of 0.6% aqueous solution of TBA were added to 0.5ml of sample; test tubes were heated at 95ºC for 15 minutes to develop the color. After a cooling period, TBARS formation was measured spectrophotometrically at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing absorbance of the control with that of test compounds.[6]

$$\% \text{ of Scavenging} = \frac{\text{Absorbance control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Superoxide Scavenging Activity: The reaction mixture contained 50mM phosphate buffer (pH 7.6), 20mg riboflavin, 12mM EDTA, NBT 0.1mg/3ml, added in that sequence The reaction was started by illuminating the reaction mixture with different concentrations of sample extract for 15minutes. Immediately after illumination, the absorbance was measured at 590nm.[7] Percentage of scavenging was measured by the above formula

Nitric oxide Radical Scavenging Activity: Sodium nitroprusside 5mM was prepared in phosphate buffer pH 7.4. To 1ml of various concentrations of extract, sodium nitroprusside 0.3ml was added. The test tubes were incubated at 25ºC for 5hours, after which 0.5ml of Griess reagent was added. The absorbance was read at 546nm. Percentage of scavenging was measured by the above formula.

DPPH Scavenging Activity: To 1ml of various concentrations of test compound, 1.0ml of 0.5mM DPPH was added. The test tubes were incubated at 37ºC for 30minutes.[1] The absorbance was read at 517nm. Percentage of scavenging was measured by the above formula.

FRAP Assay: The FRAP reagent was prepared by adding 200ml of acetate buffer, 20ml TPTZ; 20ml FeCl3;and 24ml of distilled water. To 1ml of various concentrations of test compound added 1ml of FRAP reagent, mixed the contents thoroughly and incubated at 73ºC for 5 minutes. Read the absorbance at 593nm.[9]

***In vitro* Cytotoxicity test:** EAC tumor cells from intra peritoneal cavity of tumor induced mice was aspirated out and transferred it into the tube containing PBS. These cells were washed three times with PBS. It was resuspended into known volume of PBS. Checked the viability of the cells using Trypan Blue dye exclusion method. The cell suspension (1×10^6 cells) was added into eppendorf tubes containing various concentrations of the test compound and the volume was made up to 700µl using PBS. Control tubes ere also maintained containing only cell suspension in PBS. The assay mixture was incubated at 37ºC for 3 hours. Then the percent of dead cells were evaluated by Trypan Blue dye exclusion method.

$$\text{Percentage of dead cells} = \frac{\text{Number of dead cells}}{\text{Total number of cells}} \times 100$$

Results and Discussion

The human body produces reactive oxygen species capable of oxidizing biomolecules, which can damage DNA, cells and contribute to chronic diseases. This process can be attenuated or perhaps reversed by diets that have the ability to scavenge reactive oxygen species. Hydroxyl radicals generates various products from the DNA bases which mainly include C-8 hydroxylation of guanine to form 8-oxo-7, 8 dehydro-2'deoxyguanosine, a ring opened product. ROS induced DNA damages include various mutagenic alterations in human C-Ha-ras-I proto-oncogene and to induce mutation in the p53 tumor suppressor gene. Besides, ROS may interfere with normal cell signaling, resulting thereby in alteration of the gene expression, and development of cancer by redox regulation of transcriptional factors/activator and/or by oxidatively modulating the Proteinkinase cascades.[4] Study revealed that the aqueous extract of *Cucumis melo* possess hydroxyl radical, superoxide radical and nitric oxide radical scavenging activity in a concentration dependent manner (Table 1). The scavenging activity may be due to the presence of more Vitamin C in the fruit, which is a potent scavenger of all the three free radicals.

DPPH scavenging activity showed that increased concentration of extract possess increased scavenging activity (Table 2).

The ferric reducing antioxidant power of the extract had been determined by FRAP assay (Table 3).

The *in vitro* cytotoxicity of the aqueous extract of *Cucumis melo* against Ehrlich's Ascites Carcinoma cells done at different concentrations showed cytotoxic effect against the Ehrlich's cancer cells in a dose dependent manner. As the concentration of the extract increased the cytotoxicity also increased. 50% of the cytotoxicity was observed at the concentration of 400µg. More cytotoxicity was observed at the concentration of 500µg of the extract (Graph 1, 2).

Conclusion

Since there is a tremendous historical legacy in use of plants as medicine, today more and more people take plant medicine as an alternate therapy. Free radicals are highly reactive species produced in the body during normal metabolic functions or introduced from the environment. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. It is now generally agreed that aging and age-related diseases result from ROS mediated oxidative damage of lipid, protein, nuclear and mitochondrial DNA molecules. The present study revealed that aqueous extract of *Cucumis melo* fruit pulp possess Free Radical Scavenging activity, DPPH scavenging activity , Ferric Reducing Antioxidant Power *and in vitro* cytotoxicity at different concentrations. The study can be further extended for molecular mechanism of the compounds and antitumour activity.

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Table 1: *In vitro* free radical scavenging of aqueous extract of *Cucumis melo*

S.No	Concentration of extract (mg)	Radical scavenging activity (%)		
		Hydroxyl Radical	Superoxide Radical	Nitric OxideRadical
1	20	18.8	32.7	26.9
2	40	39.1	38.5	49.0
3	60	48.1	44.1	57.2

Table 2: DPPH scavenging activity of aqueous extract of *Cucumis melo*

S.No	Concentration of extract (mg)	% of DPPH scavenged
1	20	47.2
2	60	49.7
3	100	55.0

Table 3: FRAP Assay for aqueous extract of *Cucumis melo*

EXTRACT	FRAP VALUE µm/50µl
Aqueous	240 µm

Figure1: IN VITRO CYTOTOXICITY AGAINST EAC CELLS

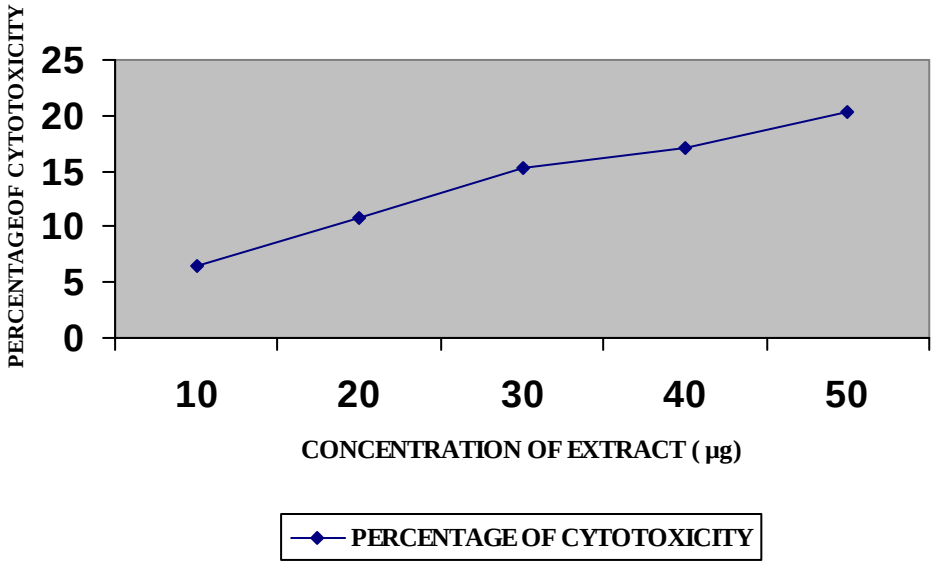

Figure 2: IN VITRO CYTOTOXICITY AGAINST EAC CELLS