



Effects of Crude Methanol Soluble Extract of *Momordica charantia* on Cancer Cell Lines and L6 Cell Line

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

A multitude of plants have been used extensively for the treatment of cancers throughout the world. In many parts of the world, especially in poor countries, this may be the only form of cancer therapy. Much research has been focused on the scientific evaluation of traditional anti-cancer drugs from the tropical plant; *Momordica charantia* (MC) is one of them and it has been used frequently as an anti-cancer agent. The green leaves, fruits, seeds and stems of *M. charantia* composed of many different proteins and steroids that are chemically active. These proteins are α and β momorcharins which possess anti-cancer and anti-HIV properties similar to crude water and methanol soluble extracts of *M. charantia*. This study investigated the anti cancer effect of crude methanol soluble extract of *M. charantia* based on dose-dependent and time-dependent on the viability of 1321N1, Gos-3, U87-MG, Sk Mel, Corl -23, Werl Rb-1 and L6 cell lines employing different concentrations of each extract or drug. The results have shown that the crude methanol soluble of *M. charantia* (200 μ g - 800 μ g) had little effect on the viability of the different cancer cell lines.

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1. INTRODUCTION

A multitude of plants have been identified and used for the treatment of different diseases throughout the world, especially in poor countries. Much research has been focused on the scientific evaluation of traditional drugs from the tropical plant; *Momordica charantia* (MC) has been commonly or frequently used as an anti-cancer agent and anti-diabetic agent and it is often described as food of medicine [1-3]. MC is commonly known as either bitter melon or bitter gourd. Bitter gourd grows in all tropical parts of the world and it is cultivated throughout South America, Asia and Africa [4].

The plant is a slender climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils [4]. It is related to squash and cucumber plants. In the Amazon, local people grow bitter melon in their gardens for food and medicines [4-6]. They add the fruit for bitter or sour flavor by parboiling it first with a dash of salt to remove some of the bitter taste [7-9]. It is used as leaf tea for the treatment of diabetes, to expel intestinal gas, to promote menstruation and as an antiviral treatment for measles, hepatitis and feverish conditions [10]. It is also used typically for sores, wounds, infections and also internally and externally to treat for worms and parasites [10-11].

MC is also used for the treatment of cancer tumours [11-13]. In the last few decades, several hundreds of studies that have been carried with MC using modern tools and they have credited MC with anti-diabetic, anti-viral, anti-tumour, anti-leukemic, anti-bacterial, anthelmintic, anti-mutagenic, anti-mycobacterial, antioxidant, anti-ulcer, anti-inflammatory and hypochlolesterolemic, hypoglyceridemic, hypotensive, immune stimulant and insecticidal properties [12-14]. MC seems to have universal medicinal properties for the treatment of different diseases. The main aim of the study is to isolate crude methanol soluble extracts of *M. charantia* using different extraction, analytic and biochemical methods.

2. MATERIALS AND METHODS

2.1 Cell Lines

1321N1 (ECACC, UK), Gos-3 (DSM2, Germany), U87-MG (ECACC, UK), L6, WERI-Rb1 (ECACC,

UK), SK-Mel (DSM2, Germany), Cori-23 (ECACC, UK).

2.2 Chemicals and Reagents

Methanol and isopropanol, hexane (Fisher Scientific, UK), Cell titer-Glo luminescent cell viability Assay kit (Promega, UK), MTS assay (Promega, UK). Fura-2 (AM) (Sigma, UK).

2.3 Extraction of Crude Methanol Soluble Extract of *M. charantia*

The unripe green fruits of *M. charantia* were obtained from the local market and cleaned and cut into small pieces and oven dried at 50°C about 48 hrs until it reached a constant weight. The dried sample was then pulverized with a grinder into fine homogenous powder, which was stored at 2°C until used. An amount 40 gm of the fine ground sample was extracted with 300 ml of methanol in a flask placed in an ultrasonic bath, containing water and the temperature was maintained at 65°C for 90 min. The sample was cooled to room temperature and centrifuged at 2000 rpm for 20 min. The suspension was removed and double filtered through Whatmann filter paper (No: 4 Whatmann, UK). The filtered sample was then transferred to the 500 ml round bottom rotating flask. The flask was then connected to the Rota evaporator machine through a clamp. The rotating flask was then heated by partial emersion in a hot water bath at a temperature of 50°C. A typical 100 rpm speed was used for the flask rotation. The Rota evaporated sample was then scrapped using spatula and stored at 2°C until used [15].

2.4 Passaging of the Cancer Cell Lines and Control Cell Line

The culture medium, phosphate buffer solution (PBS), and trypsin (sterile) were removed from the fridge at 4°C and subsequently placed in the water bath at 37°C for 30 min in order to equilibrate. The Laminar flow hood was turned on for 15 min, prior to start of the experiment, in order to purge the air inside the cabinet and to reach the maximum cleanliness.

The different cancer and normal cell lines were incubated at 37°C incubator in an atmosphere of 5% CO₂ in air. The cells were examined under the inverted contrast microscope to note the both confluence and general health of the cells. The

flask was passaged when the cells had reached 70-80% confluence.

The medium was aspirated from the cultured flask and was washed with sterile PBS (5 ml if 75 cm² flask and 2 ml if 25 cm² flask) in order to remove any traces of serum from the cells. This prevented the serum from inactivating the trypsin which was used to detach adherent cells from the cell clump. Trypsin solution (2 ml if 75 cm² flask or 1 ml if 25 cm² flask) was pipetted in the flask and incubated at 37°C in an incubator in an atmosphere of 5% CO₂ in air for 3-5 mins until the cells began to detach. The detachment was confirmed by observing at intervals under an inverted microscope. The cells were left in the trypsin solution for the correct length of time. If the cells were left for a longer period of time then this would lead to damage of the cells. A volume of 3 ml complete growth medium was then added to the flask to inactivate the trypsin and the cells were pipetted up and down to break up any large cell aggregates. The cell suspension was transferred from flask into 15 ml centrifuge tube and centrifuged at 1000 rpm for 5 min. Following centrifugation, the supernatant was aspirated and the cells were pellet at the bottom of the centrifuge tube. Based upon the cell pellet density volumes of 1 ml to 3 ml fresh medium were suspended in the centrifuge tube. The cell pellet was flicked properly in the medium containing 20 µl of trypsinised cell suspension and 80 µl of trypan blue (used to detect dead cells in the cell suspension 1:5 ratio). The contents were mixed well together and a haemocytometer test was performed using 1 ml of cell suspensions. This process helped to assess the total number of the cell suspension present in the centrifuge tube and which was required to make 1 or 2 flasks and to do 96 well plates. Thereafter, the cells were frozen in liquid nitrogen depending on the number of cells present per ml. The cell suspension was divided in either one or several flasks (depending on the cell density) and fresh growth medium (10 ml to 12 ml if 75 cm² flask and 5 ml if 25 cm² flask) was added to the flasks. These were then placed in a 5% CO₂ incubator to continue cell growth.

3. CELL VIABILITY EXPERIMENTS

3.1 Preparation and Application of Crude Methanol Soluble Extracts of *M. charantia* on the Cancer and L6 Cell Lines

An amount of 30 mg of the crude methanol - soluble extract of *M. charantia* was initially

dissolved in 500 µl of phosphate buffer by continuous stirring and with the brief use of a sonicator water bath. This was made up to 5 ml by adding 4.5 ml of the cell medium. The methanol-soluble crude extract stock solution was transferred to a 10 ml syringe and sterile filtered using 0.22 µm filters into other sterile 10 ml Universal bottles. These stock solutions were stored in a sealed tube in the fridge until required. Once removed from the fridge, the prepared crude methanol -soluble extract of *M. charantia* solutions were gently warmed in water bath at 37°C in order to ensure that the methanol soluble crude extract was mixed complete in solution, before aliquoting. Volumes of 34 µl, 68 µl, 102 µl, 136 µl contained 200 µg, 400 µg, 600 µg, and 800 µg of the crude methanol -soluble extract of *M. charantia* respectively. Different concentration of methanol soluble crude extract in cell medium was transferred in triplicate using a Gilson pipette to 96 well plates to give a final volume of 200 µl to the treated cell wells. An equivalent volume of 200 µl of the medium was added to the control (untreated) well with cells. In another series of control experiments, the different cancer cell lines were incubated in 96 well plates for the same period but in the absence of methanol extract of *M. charantia*. Following every 6 hrs of incubation, the plate for either test and control was removed from incubator for 30 min. MTS procedure was carried out in the transparent 96 well plates (Griner, UK). In this study, both time course and dose-dependent experiments were performed. The time-course experiments were done over a period of 48 hours, where the dose dependent experiments were done during incubation period of 24 hrs.

3.2 Time-course Experiments

Cell suspensions of either 1321N1, Gos-3, U87-MG, Sk Mel, Corl -23, Weri Rb-1 or L6 obtained during passaging were diluted (1:10 ratio). The required volumes of cell suspensions and fresh medium volumes were calculated based on the number of wells to be plated. The dilution was done such that 200 µl of each suspension contained 2500 cells. After seeding the cells, the plates (transparent 96 well plates) were incubated for 6, 12, 18, 24 and 48 hrs at 37°C in a 5% CO₂ incubator. After each time period the media from 96 well plates were tipped out completely on sterile tissue paper. The drug stock solution was transferred to a 10 ml syringe and sterile filtered using 0.22 µm filters into another sterile 15 ml centrifuge tube. A volume of

100 μ l of cell medium (sterile filtered) contains 800 μ g of crude methanol soluble extract of *M. charantia* was then added to the 96 well plates. The 96-well plates were incubated for 6, 12, 18, 24 and 48 hours at 37°C in a 5% CO₂ incubator.

In another series of control experiments, the different cancer cell lines were incubated in 96 well plates for the same period but in the absence of any extract of *M. charantia*. Following every 6 hrs of incubation, the plate for either test and control was removed from incubator for 30 min. MTS procedure was carried out in the transparent 96 well plates (Griner, UK). A volume of 20 μ l of MTS assay sample was added to wells containing both treated and untreated cells. The ratio resulted in a final concentration of 317 μ g/ml MTS assay in the assay wells. Each plate was then placed in the incubator at 37°C in a 5 % CO₂ atmosphere for 90-120 min. The absorbance's was measured at a 492 nm wavelength, and 30 sec of shaking time and 30 sec for the settling time in the plate reader. The absorbances of the crude extract alone at different time points in the medium were also measured as background sequence. These values were subtracted from the test values at the different time points. The same was also done for the dose-dependent experiments or a combination of extracts with drug.

3.3 Dose-dependent Experiments

In this series of experiments, the experimental procedure was the same except that the test cells were incubated with different concentrations of each extract for 24 hrs. Initial experiments established maximum cell viability at 24 hrs of incubation with the crude methanol-soluble extract of *M. charantia*.

Cell suspensions of either 1321N1, Gos-3, U87-MG, Sk Mel, Corl-23, Weri Rb-1 or L6 obtained during passaging were diluted (1:10 ratio). The required volume of cell suspension and fresh medium volume were calculated based on number of wells to be plated. The dilution was done such that 200 μ l of the suspension contained 2500 cells. After seeding the cells, the plates (transparent 96 well plates) were incubated for 24 hrs at 37°C in a 5% CO₂ incubator. Control 96 well plates were incubated for 24 hrs in the absence of extracts. After 24 hrs the media from 96 well plates were tipped out completely on sterile tissue paper. The drug stock solution was transferred to a 10 ml syringe and sterile filtered using 0.22 μ m filters into

another sterile 15 ml centrifuge tube. These stock solutions were stored in a sealed tube in the fridge until required. A volume of 100 μ l of cell medium (sterile filtered) contained extract concentrations of 200 μ g, 400 μ g, 600 μ g, 800 μ g in the 96 well plates. After the supplement of the crude extract, the 96-well plate was again incubated for 24 hrs at 37°C in a 5% CO₂ incubator. Following 24 hrs of incubation, each plate was removed from incubator for 30 min. MTS procedure was carried out in the transparent 96 well plates (Griner, UK). A volume of 20 μ l of MTS assay sample was added to wells containing both treated and untreated cells. The ratio resulted in a final concentration of 317 μ g/ml MTS assay in the assay wells. The plate was then placed in the incubator at 37°C in 5 % CO₂ atmosphere for 90-120 min. The absorbance was measured at a 492 nm wavelength by initial 30 sec of shaking time and 30 sec of settling time of the plate in the plate reader.

4. RESULTS AND DISCUSSION

The untreated (no methanol extract) cell line for each (first bar chart) is also shown as 100% in the Fig. 1 for comparison. Each cell line was incubated with crude methanol soluble extract of *M. charantia* for 24 hrs. Similarly, each control cell line was incubated in the medium alone for 24 hrs. Data are mean \pm SD, n=6 different experiments in duplicate; *p>0.05 for untreated (100 % viability) compared to the treated cells for the different concentrations except for 1321N1 in which *p<0.05 for 600 μ g and 800 μ g compared to control.

The time-course effects of 800 μ g of crude methanol soluble extract of *M. charantia* on the viability of six different cancer cell lines (1321N1, Gos-3, U87-MG, Sk Mel-2, Corl-23, Weri Rb-1) and on healthy L6 muscle cell line for comparison shown as 100% in the Fig. 2. Cell lines incubated at the same time point without any extract are also shown in the figure for comparison. Solid straight line shows the 100 % values for each time point. Cell viability for each time point was expressed as percentage of the respective control (no extract, but only cells in medium) at each time point of incubation. Each cell line was incubated with the crude water-soluble extract of *M. charantia* for up to 48 hrs. All Data are mean \pm SD, n=6 different experiments in duplicate; *p<0.05 for all test samples compared to control (100%).

A number of preliminary studies have shown anti-cancer activity of *M. charantia* against lymphoid, leukaemia, lymphoma, choriocarcinoma, melanoma, breast, skin and prostatic cancers [14-16]. Maximal anti-carcinogenic activity was demonstrated following chronic treatment with the hot water extract of *M. charantia* in uterine adenomyosis and mammary tumour growth in mice [16-17].

The beneficial anti-cancer effects of the crude water-soluble extract of *M. charantia* have been

reported previously by several other investigators [16-18]. Moreover, a number of preliminary studies have been conducted using the crude preparation of *M. charantia*. In all these studies, the chemical profile of the extract was not reported in the investigations. Nevertheless, some studies have demonstrated marked biological activities of several compounds including charantin, MAP 30, momordicin, alpha, beta momorcharin extracted from *M. charantia* [19-21].

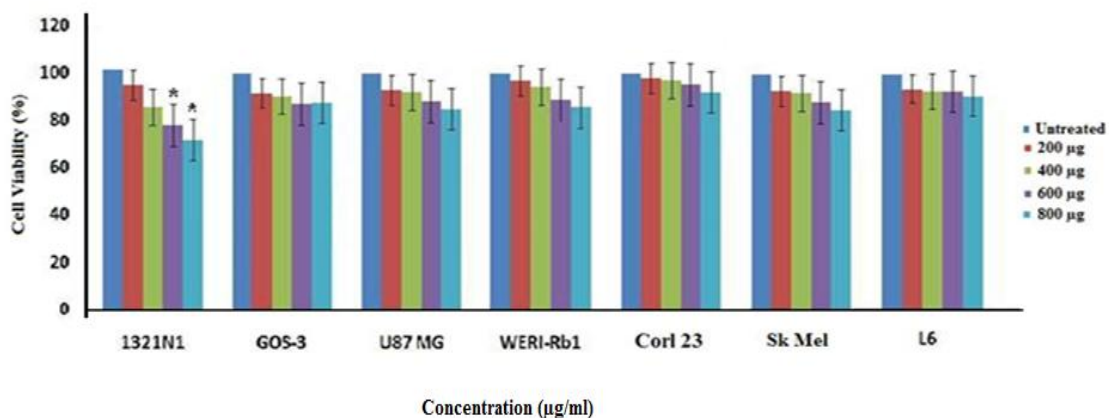


Fig. 1. Bar charts showing the effects of different concentrations (200-800 µg/ml) of the crude methanol soluble extract of *M. charantia* on the viability of six different cancer cell lines (1321N1, Gos-3, U87- MG, Sk Mel-2, Corl -23, Weri Rb-1) and healthy L6 muscle cell line for comparison

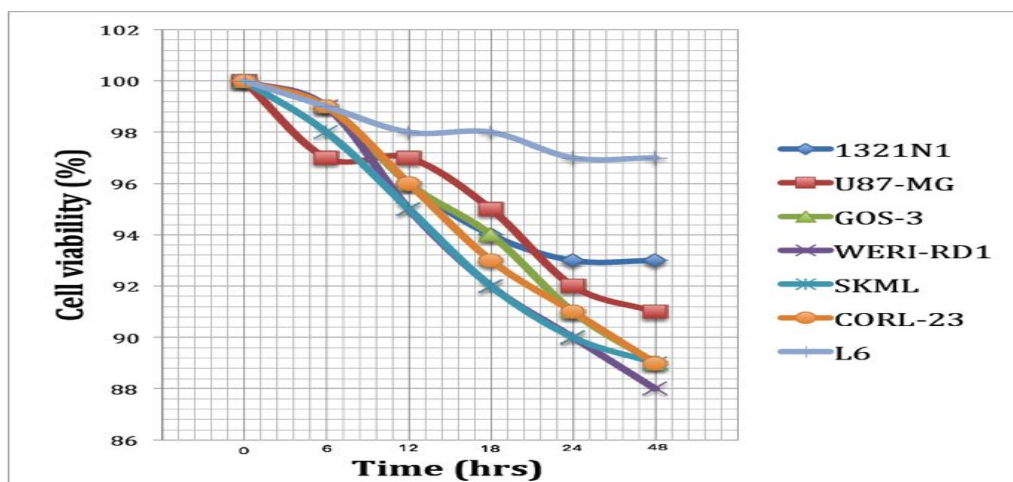


Fig. 2. The time-course effects of 800 µg/ml of crude methanol soluble extract of *M. charantia* on the viability of six different cancer cell lines (1321N1, Gos-3, U87-MG, Sk Mel-2, Corl-23, Weri Rb-1) and on healthy L6 muscle cell line for comparison

5. CONCLUSION

In this study, the methanol soluble extract of *M. charantia* failed to elicit any decrease in the viability of each cancer cell line except for a small decrease in the viability of 1321N1 cell line, but only at high concentrations. This may be due to the fact that the methanol extraction denatures the active ingredient, which was less effective in decreasing cell viability (cell death). However, further experiments are required to investigate the sub-cellular mechanisms associated to cell death including the involvement of kinases and gene expressions for apoptotic mediators.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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