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EFFECTS OF ALPHA, BETA MOMORCHARIN FRUIT EXTRACT WITH THE COMBINATION OF VINBLASTINE IN THE TREATMENT OF GLIOMA CANCER IN-VIVO

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ABSTRACT

The vegetable Momordica charantia L., (family: Cucurbitaceae) is a scientific name of the plant and its fruit. It is also known by other names, for instance in the USA it is known as Bitter gourd or balsam pear while it's referred to as the African cucumber in many African countries. M.charantia is believed to posse's anti-carcinogenic properties and it can modulate its effect via xenobiotic metabolism and oxidative stress. This study was specifically designed to investigate the cellular mechanisms where by α, β momorcharin an extract of M. charantia can induce cell death with the combination of Vinblastine. Different concentration (200µM - 1000µM) of the α, β momorcharin fruit extract were treated (24 hrs incubation) separately with three different cancer cell lines 1321N1, Gos-3, U87-MG and normal L6 muscle cell line. The results also show that Vinblastine (250 µg) with (1000 µM) of the α, β momorcharin extract of M. Charantia, and result in significant decreases in cell viability for each cell line, these effects were additive compared to the individual effect of Vinblastine.

KEYWORDS: Cancer cell lines, α, β momorcharin extract of M. Charantia and Vinblastine (VBL).

INTRODUCTION

The water-soluble extract of the M. charantia can significantly reduce blood glucose concentrations in type-1 diabetic rats.[1] Several studies have reported that the water-soluble extract of M. charantia can exert anti-cancerous activity through inhibition of DNA, RNA and cellular protein synthesis.[1-5] The fruit juice of M. charantia has been found to increase glucose up take by several tissues in vitro and moreover, it can increase the storage of glycogen by the liver.[5-6] Vinblastine is a natural product with antitumor activity.[7-8]

Vinblastine is used in combination with other chemotherapy drugs to treat Hodgkin's lymphoma (Hodgkin's disease) and non-Hodgkin's lymphoma (types of cancer that begin in a type of white blood cell that normally fights infection), and cancer of the testicles.[8-9] It is also used to treat Langerhans cell histiocytosis (histiocytosis X; Letterer-Siwe disease; a condition in which too many of a certain type of white blood cell grows in parts of the body).[10-13] It may also be used to treat breast cancer that has not improved after treatment with other medications and gestational trophoblastic tumors (a type of tumor that forms inside a woman's uterus while she is pregnant) that has not improved after surgery or treatment with other medications. Vinblastine is in a class of medications called vinca alkaloids. It works by slowing or stopping the growth of cancer cells in your body.

MATERIALS AND METHODS

Extraction method for either of α or β momorcharin

The whole fruit of bitter gourd was ground and homogenized in 2 mM sodium phosphate buffer, pH 7.5. The resulting slurry was then stirred for 3 hrs to extract the crude proteins. The insoluble component from crude proteins was removed by the filtration and centrifugation at 30,000 x g for 1 hour at 48°C. By using 2 mM sodium phosphate buffer, pH 7.5, the crude protein solution was dialysed. The dialysed protein sample was applied to DEAE Sepharose column equilibrated with 2 mM sodium phosphate buffer at pH 7.5. The unbound proteins were then applied to Mono-S column which was equilibrated by 2 mM sodium phosphate buffer at pH 7.5 and eluted by 0.5 m of NaCl. The fraction corresponding to either alpha and beta or alpha, beta momorcharin, which was confirmed the N-glycoside activity RNA, was concentrated and dialysed against 20 mM Tris-HCl buffer, pH 7.8. The chromatography was performed on Bio Logic DuoFlow system (BioRad, Hercules, CA) at 48°C. The purity of α and β momorcharin was examined by SDS-PAGE and gel filtration chromatography. The

concentration of α and β momorcharin was determined by spectrophotometrically using optical absorbance at 280nm.

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% CO_2 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 cm^2 flask and 2 ml if 25 cm^2 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 cm^2 flask or 1 ml if 25 cm^2 flask) was pipetted in the flask and incubated at 37°C in an incubator in an atmosphere of 5% CO_2 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 tryphan 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 cm^2 flask and 5 ml if 25 cm^2 flask) was added to the flasks. These were then placed in a 5% CO_2 incubation.

Preparation and application of α, β momorcharin extracts of M. Charantia on the cancer and L6 cell lines

Amounts of 14.51 mg, 29.25 mg, 43.53 mg, 58.50 mg, and 72.57 mg of either alpha or beta momorcharin and alpha, beta momorcharins (9.7 kDa) were weighed out separately in 5 ml universal vials and initially dissolved in 500 µl of phosphate buffer by continuous stirring and with the brief use of a sonicator water bath. These were then made up to 5 ml by adding 4.5 ml of the cell medium to give concentrations 200µM, 400µM, 600µM, 800µM, 1000µM, respectively. The drug (extract) stock solution was transferred to a 10 ml syringe and sterile filtered using 0.22 µm filters into another 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 drug (extract) solutions were gently warmed in water bath at 37°C in order to ensure that the alpha, beta momorcharin was in a complete solution, before aliquoting. Volumes of 40 µl, 80µl, 120µl, 160µl, 200µl contained 200µM, 400µM, 600µM, 800µM, 1000µM, respectively. Different concentrations of alpha beta momorcharins were transferred in triplicate using a Gilson pipette to 96 wells plate and the volume made to 200 µl by adding the cell media to both treated and control cell wells. Both control (untreated) and treated with (alpha beta momorcharin) 96 well plates were incubated for 24 hrs.

Dose dependent effects of Vinblastine on cancer cell line viability

In this series of experiments, different cancer cell lines (1231N1, Gos-3, U87-MG) and healthy L6 muscle cell line were incubated with the different concentrations of Vinblastine (50 - 250 µg) for 24 hours. Control cell lines were also incubated for the same period of time but without any Vinblastine. At the end of the incubation period, cell viability of each cell line was measured using the MTS assay.

Combined effects of either Vinblastine with the α, β momorcharin extract of M. charantia.

Different cancer cell lines (1231N1, Gos-3, U87-MG) and healthy L6 muscle cell line were incubated either with Vinblastine (250 µg) and the α, β momorcharin extract of M. charantia (1000 µM) or Vinblastine (250 µg) and 1000 µM) of the alpha, beta momorcharin for 24 hours. Control cell lines were also incubated for the same time but without any drug or extract. At the end of the incubation period, cell viability of each cell line was measured using the MTS assay.

Statistical Analysis

All control and test data collected from the different experiments were analysed using Statistical Package for Social Sciences (SPSS) version 17, Student's t- test and ANOVA test. Data obtained were expressed as mean ± standard deviation (S.D). Each experiment was repeated for 4-6 times in duplicate (6 for cell viability and 4 for

cell signalling) to ensure the accuracy of results. A value of ($p < 0.05$) was taken as significant.

RESULTS AND DISCUSSION

Dose-dependent effects of Vinblastine on cell viability

Figure 1 shows the effects of different concentrations (50 - 250 µg) of Vinblastine on the viability of the three different cancer cell lines and on healthy L6 skeletal muscle cell line employed in this study. Also shown in the figure 1 are the untreated three different cancer cell lines and healthy L6 skeletal muscle cell line for comparison. All the cells were treated with Vinblastine for 24 hours. Each control cell lines were also incubated for 24 hrs but with no Vinblastine. The results show that in all three different cancer cell lines (1321N1, Gos-3, U87-MG), Vinblastine can evoke marked and significant ($p < 0.05$) decreases in the cell viability (cell death) compared to untreated cells (100% viability). These effects of the Vinblastine were dose-dependent with maximal cell death occurring with 250 µg. Similarly, Vinblastine significantly ($p < 0.05$) decreased the viability of healthy L6 skeletal muscle cell line compared to untreated L6 cell line but mainly at a high dose. The results also show that Vinblastine was more effective in killing 1321N1 and Gos-3, cell lines. It has less effective on U87-MG cell line, which seems to be more resistant to the drug. The surprised finding in this study was that Vinblastine could also kill healthy L6 skeletal muscle cell compared to the α, β momorcharin extract of M. charantia, which had no detectable effect on the viability of L6 cell line.

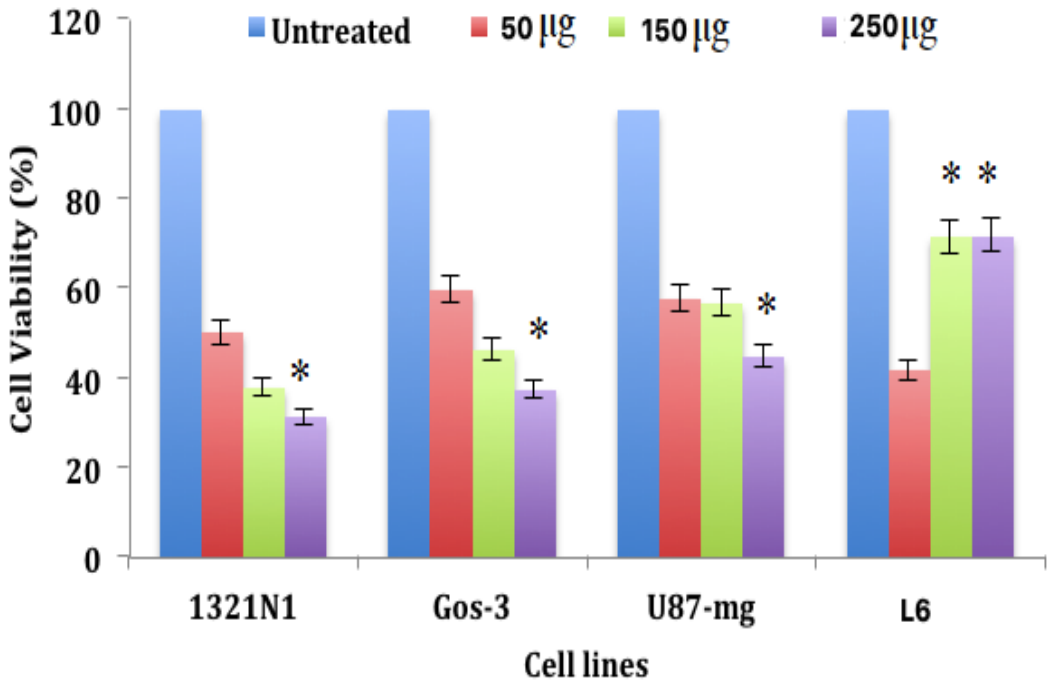

Fig. 1: Dose-dependent effects of Vinblastine.

Combined effects of α, β momorcharin extract of M. charantia with Vinblastine

Figure 2 shows the effect of Vinblastine (250 µg) alone or the α, β momorcharin extract of M. charantia (1000 µM, a high dose) alone or a combination of Vinblastine (250 µg) with the α, β momorcharin extract of M. charantia (1000 µM) on the viability of the three different cancer cell lines and on healthy L6 skeletal muscle cell line employed in this study. Also shown in the figure 2 are the untreated three different cancer cell lines and healthy L6 skeletal muscle cell line for comparison. All the cells were treated with Vinblastine and α, β momorcharin extract of M. charantia (drug + extract) for 24 hours. Control cell lines were also incubated for the same time. The results show that in all three different cancer cell lines (1321N1, Gos-3, U87-MG,) either Vinblastine or α, β momorcharin extract of M. charantia can evoked marked and significant $p < 0.05$ decreases in the cell viability (cell death) compared to untreated cells (100% viability). However, when Vinblastine was combined with the α, β momorcharin extract of M. charantia, there was a further decrease in cell viability. These values were significantly ($p < 0.05$) different compared to either untreated cell (100%) or cell treated with either Vinblastine or α, β momorcharin extract of M. charantia.

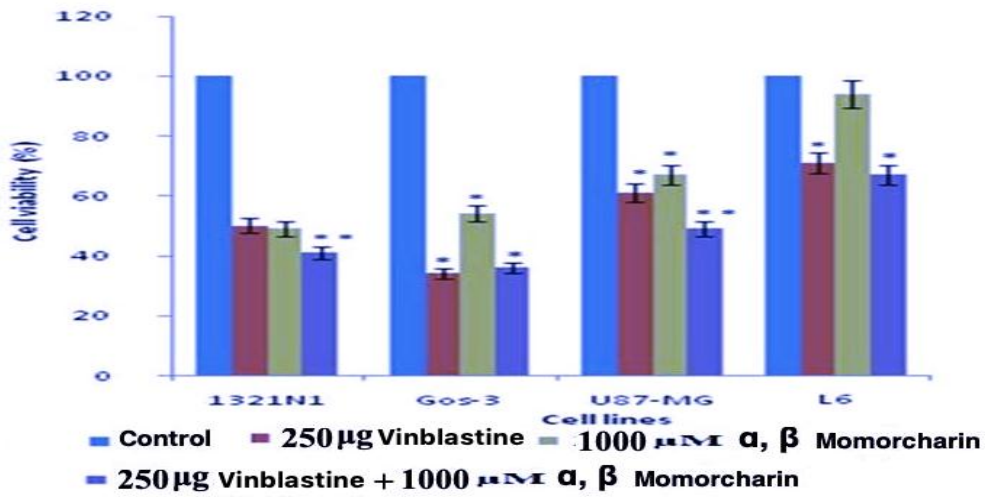

Fig. 2: Effect of either of 250 µg Vinblastine alone or 1000 µM of α, β momorcharin of M. charantia alone and combination of Vinblastine (250 µg) with α, β momorcharin extract of M. charantia (1000 µM) on the viability of three different cancer cell lines.

Similarly, Vinblastine combined with the α, β momorcharin extract of M. charantia can evoke significant ($p < 0.05$) decrease in the death of healthy L6 skeletal muscle cell line. The results also show that combined drugs (drug + extract) were more effective in killing 1321N1, Gos-3, cell lines. It has less effective on U87-MG cell lines.

This study employed the α, β momorcharin extracts of M. charantia, and commercially available anti-cancer drugs Vinblastine to investigate their effects on the viability (cell death) of three different cancer cell lines compared to healthy L6 skeletal muscle cell line. Either the α, β momorcharin extract of M. charantia, Vinblastine was tested alone measuring the viability of each cell line. In some experiments, Vinblastine was combined with α, β momorchar in extracts of M. charantia, to investigate any potentiating or attenuating effect on cell viability. The rationale for this study was that M. charantia, a local plants-base (herbal) medicine could be used to treat different types of cancers. The results of the present study have shown that either Vinblastine can significantly decrease the viability of 1321N1, Gos-3, U87-MG, cancer cell lines. Both anti-cancer drugs also decreased the viability of healthy L6 skeletal muscle cell line. The effect of each drug was dose–dependent with maximal effect occurring at Vinblastine. The results of this study also show that combining a moderate to a high dose of Vinblastine with a high dose of either the α, β momorcharin extract of M. charantia only produce a small, but significant decrease in the viability of each cancer cell line compared to the effect of Vinblastineand the α, β momorcharin extract of M. charantia alone. This small decrease in cell viability of each cell line was slightly significant, but it was neither additive nor synergetic compared to the separate effect of each. This was a rather surprising result in this study.

CONCLUSION

In conclusion, the results of this study have clearly demonstrated that the α, β momorcharin extract of M. charantia can evoke significant decreases in cancer cell viability (an increase in cell death) without killing healthy cell line like L6 skeletal muscle cell line. Either Vinblastine with maximal effect of Vinblastine can also elicit dose-dependent decreases in cancer cell viability. Combining Vinblastine with either the α, β momorcharin extract of M. charantia had no additive or synergetic effect on the viability of each cell line compared to the effect of either alone. It is concluded that extracts of M. charantia possess anti-cancer properties since they can induce cell death.

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