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Original Research Article

Effects of *Momordica charantia* fruit extract with the combination of paclitaxel 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 its 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. Different concentration (200µg - 800µg) of the crude water soluble fruit extract were treated (24 hrs incubation) separately with five different cancer cell lines 1321N1, Gos-3, U87-MG and normal L6 muscle cell line. The results also show that paclitaxel (250 µg) with (800 µg) of the crude water- soluble extract of *M. Charantia*, result in significant decreases in cell viability for each cell line, these effects were additive compared to the individual effect of paclitaxel**.**

Keywords: Cancer cell lines, crude water-soluble extract of *M. Charantia* and paclitaxel (PAT).

INTRODUCTION

The water-soluble extract of the *M. charantia* can significantly reduce blood glucose concentrations in type-1 diabetic rats (Ahmed I, 1999). 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 (Licastro F *et al* 1980 Zhu Z J, 1990, Tsao SW *et al.*, 1990, Asli S, 2007). 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 (Manoharan G *et al.*, 2011, Manoharan G *et al.*, 2014). Paclitaxel is a natural product with antitumor activity. Paclitaxel is a diterpene alkaloid derived from the dried bark of Pacific yew tree Taxus brevifolia (Moore MJ *et al.*, 1998, Aoki T *et al.*, 2003). The chemical name for paclitaxel is 5β,20-Epoxy-l,2α,4,7β,10β,13α-hexahydroxytax-l l-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine. Paclitaxel is a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.9 g/mol (Brada M, 2002, Espinosa E *et al.*, 2003). Paclitaxel is highly lipophilic, and melts at around 216-217°C and is used in treatment of various advanced carcinomas such as ovarian cancer, lung cancer, breast cancer, acute leukemias, head and neck cancers and Kaposi's sarcoma (Schwartzbaum JA *et al.*, 2006, Sun Y *et al.*, 2001, Fewer D *et al.*, 1972). Paclitaxel is a mitotic inhibitor that blocks the proliferation of cancer celland is commercially available as Taxol® that consists of Paclitaxel solubilised in Cremophor EL (polyethoxylated castor oil) and dehydrated alcohol (1:1v/v) (Friedberg EC, 2001) Unfortunately, severe adverse reactions like hypersensitivity reactions are caused by Cremophor EL (Tonn JC *et al.*, 1994, Sawyer JR *et al.*, 1991, Schwartzbaum JA *et al.*, 2006, Omar S, 2007).

MATERIALS AND METHODS

Extraction of crude water-soluble extract of *M. charantia*

The unripe green intact fruits of *M. charantia* were obtained from the local supermarket and subsequently cleaned and cut into small pieces. Approximately, one kilogram of chopped green fruit was liquidized in distilled water for 5-10 min using a blender. The juice was then kept in a hot water bath for 2 hours at the temperature of 67°C. The fruit juice was centrifuged at 5000 RPM (Beckman, UK) for 30 min. The suspension was removed and filtered through Whatmann filter paper (No: 4 Whatmann, UK). The filtered green sample (supernatant) was then transferred to the 1000 ml round bottom rotating flask. The flask

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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 40°C. A typical 120-rpm speed was used for the flask rotation. The Rota evaporated sample was then scrapped using spatula and dried overnight in an oven at 43°C. This crude water-soluble extract (powder) was stored at 2°C for further use.

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 three 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 crude water-soluble extracts of *M. Charantia* with paclitaxel on the cancer and L6 cell lines.

An amount of 30 mg of the crude water-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 water-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 water-soluble extract of *M. charantia* solutions were gently warmed in water bath at 37°C in order to ensure that the water-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 water-soluble extract of *M. charantia* respectively. An equivalent volume of 200 µl of the medium was added to the control (untreated) well with cells. In this study, both time course and dose-dependent experiments were performed.

Dose dependent effects of paclitaxel 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 paclitaxel (50 - 250 µg) for 24 hours. Control cell lines were also incubated for the same period of time but without any paclitaxel. 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 paclitaxel on cell viability

Figure 1 shows the effects of different concentrations (50 - 250 µg) of paclitaxel 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 paclitaxel for 24 hours. Each control cell lines were also incubated for 24 hrs but with no paclitaxel. The results show that in all three different cancer cell lines (1321N1, Gos-3, U87-MG), paclitaxel 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 paclitaxel were dose-dependent with maximal cell death occurring with 250 µg. Similarly, paclitaxel 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 paclitaxel 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 paclitaxel could also kill healthy L6 skeletal muscle cell compared to the crude water-soluble extract of *M. charantia, which* had no detectable effect on the viability of L6 cell line.

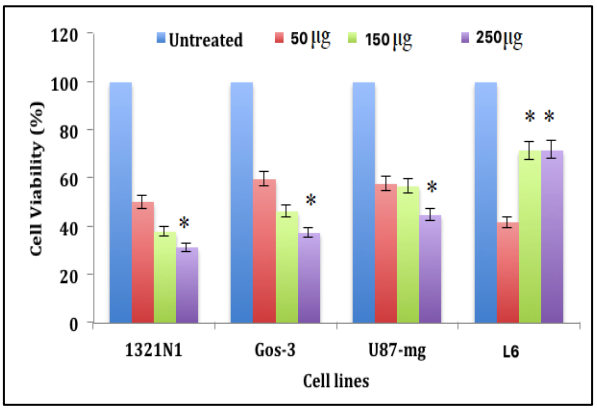

Fig. 1: Dose-dependent effects of paclitaxel

The combined effects of crude water-soluble extract of *M. charantia* with paclitaxel

Figure 2 shows the effect of paclitaxel (250 µg) alone or the crude water-soluble extract of *M. charantia* (800 µg, a high dose) alone or a combination of paclitaxel (250 µg) with the crude extract soluble extract of *M. charantia* (800 µg) 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 both paclitaxel and the crude water-soluble extract of *M. charantia* (drug + crude 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 paclitaxel or crude water-soluble 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 paclitaxel was combined with the crude water-soluble extract of *M. charantia*, there was a further decrease in cell viability. These values were significantly ($p < 0.05$) different compared to either untreated cells (100%) or cell treated with either paclitaxel or crude water-soluble extract of *M. charantia.*

Similarly, paclitaxel combined with the crude water-soluble 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 + crude extract) were more effective in killing 1321N1, Gos-3, cell lines. It has less effective on U87-MG cell lines.

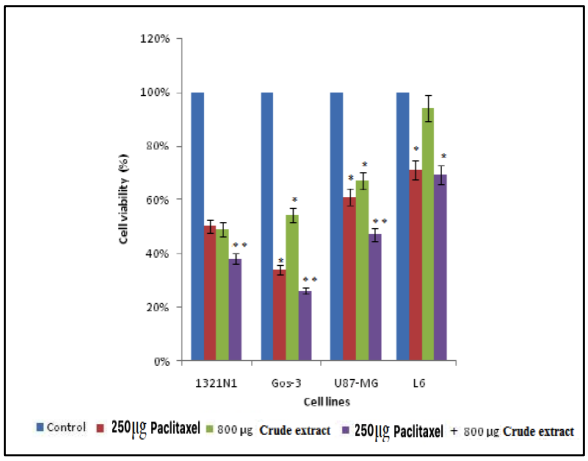

Fig. 2: Effect of either of 250 µg paclitaxel alone or 800 µg of crude water-soluble extract of *M. charantia* alone or a combination of paclitaxel (250 µg) and the crude extract soluble extract of *M. charantia* (800 µg) on the viability of three different cancer cell lines

This study employed the crude water soluble extracts of *M. charantia*, and commercially available anti-cancer drugs paclitaxel 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 crude water-soluble extract of *M. charantia*, paclitaxel was tested alone measuring the viability of each cell line. In some experiments, paclitaxel was combined with crude water-soluble 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 paclitaxel 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 paclitaxel. The results of this study also show that combining a moderate to a high dose of paclitaxel with a high dose of either the crude water-soluble extract of *M. charantia* only produce a small, but significant decrease in the viability of each cancer cell line compared to the effect of paclitaxel the crude water-soluble 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 crude water-soluble 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 paclitaxel with maximal effect of paclitaxel can also elicit dose-dependent decreases in cancer cell viability. Combining paclitaxel with the crude water-soluble 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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REFERENCES

1. Ahmed, I., Sharma, A. K., Ponery, A. S., Bener, A., & Singh, J. (1999). The influence of *Momordica charantia* fruit extract on ultrastructural abnormalities of myelinated fibres in experimental diabetes. *International Journal of Diabetes*, *7*(3), 110-121.
2. Licastro, F., Franceschi, C., Barbieri, L., & Stirpe, F. (1980). Toxicity of*Momordica charantia* lectin and inhibitor for human normal and leukaemic lymphocytes. *Virchows Archiv B*, *33*(1), 257-265.
3. Zhu Z J. (1990). Studies on the active constituents of *Momordica charantia*. Yao Hsueh. Hsueh, 25(3), 898-903.
4. Tsao, S.W., Ng, T.B, & Yeung, H.W. (1990). Toxicities of trichosanthin and alpha-momorcharin, abortifacient proteins from Chinese medicinal plants, on cultured tumor cell lines. Toxicon, 28(4), 1183-1192.
5. Asli, S., & Alaattin, S. (2007). Antioxidant and chemo protective properties of *Momordica charantia* L. (bitter melon) fruit extract. Afrcan Journal of Biotechnology, 3(6), 273-277.
6. Manoharan, G., & Singh, J. (2011). The Anti-Diabetic Effects of *Momordica charantia*: Active Constituents and Modes of Actions. The Open Medicinal Chemistry Journal, 5(6),70-77.
7. Manoharan, G., & Singh, J. (2014). Effect of α, β momorcharin on viability, caspase activity, cytochrome c release and on cytosolic calcium levels in different cancer cell lines. Molecular and Cellular Biochemistry, 2(6), 4233-240.
8. Moore, M.J., Feld, R., Hedley, D., Oza, A., & Siu, L.L. (1998). A phase II study of temozolomide in advanced untreated pancreatic cancer. Invest New Drugs, 16(1), 77-79.
9. Aoki, T., Mizutani, T., Ishikawa, M., Sugiyama, K., & Hashimoto, N. (2003). A first feasibility study of temozolomide for Japanese patients with recurrent anaplastic astrocytoma and glioblastoma multiforme. Int.J.Clin.Oncol, 8(6), 301-304.
10. Brada, M. (2002). NICE verdict on Temozolomide: where next? Br. J.Cancer, 86(6), 499-500.
11. Espinosa, E., Zamora, P., Feliu, J., & Baron, M.G. (2003). Classification of anticancer drugs- a new system based on therapeutic targets. Cancer treatment Reviews, 29(11), 515-523.
12. Schwartzbaum, J.A., Fisher, J.L., Aldape, K.D., & Wrensch M. (2006). Epidemiology and molecular pathology of glioma. Nature Clinical Practice Neurology, 2(6), 494-503.
13. Sun, Y., Huang, P. L., Li, J. J., Huang, Y. Q., Zhang, L., Huang, P. L., & Lee-Huang, S. (2001). Anti-HIV agent MAP30 modulates the expression profile of viral and cellular genes for proliferation and apoptosis in AIDS-related lymphoma cells infected with Kaposi's sarcoma-associated virus. *Biochemical and biophysical research communications*, *287*(4), 983-994.
14. Fewer, D., Wilson, C. B., Boldrey, E. B., Enot, K. J., & Powell, M. R. (1972). The chemotherapy of brain tumors: Clinical experience with carmustine (BCNU) and vincristine. *Jama*, *222*(5), 549-552.
15. Friedberg, E.C. (2001). How nucleotide excision repair protects against cancer. Nature Reviews Cancer, 1(6), 22-23.
16. Tonn, J. C., Schachenmayr, W., & Kraemer, H. P. (1994). In vitro chemosensitivity test of malignant gliomas: clinical relevance of test results independent of adjuvant chemotherapy. *Anticancer research*, *14*(3B), 1371-1375.
17. Sawyer, J. R., Swanson, C. M., Roloson, G. J., Longee, D. C., Boop, F. A., & Chadduck, W. M. (1991). Molecular cytogenetic analysis of a medulloblastoma with isochromosome 17 and double minutes. *Cancer genetics and cytogenetics*, *57*(2), 181-186.
18. Schwartzbaum, J. A., Fisher, J. L., Aldape, K. D., & Wrensch, M. (2006). Epidemiology and molecular pathology of glioma. *Nature Reviews Neurology*, *2*(9), 494.
19. Omar S. (2007). Hypoglycemic effect of the seeds of *Momordica charantia*. Fitoterapia, 78(6), 46-47.