ORIGINAL RESEARCH PAPER

Homeopathy

KEY WORDS: Momordica charantia, Homeopathy, cytotoxicity, in vitro

CYTOTOXICITY ANALYSIS OF *MOMORDICA CHARANTIA* D5 IN KERATINOCYTES, OSTEOSARCOMA AND MESENCHYMAL STEM CELLS

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ABSTRACT

Momordica charantia has been used in folk medicine since ancient times, showing excellent efficacy when properly prescribed. However, like all medications, the dose used is essential to avoid possible poisoning in patients. Therefore, homeopathy is presented as a therapeutic tool, as it reduces the risk of direct toxicity to the patient while maintaining its medicinal properties, as described in homeopathic pharmacopeias. In addition to the diseases for which it is popularly used, Momordica has been tested in homeopathic dilutions by research groups regarding its activity in cancer cells. Assessing in vitro cell viability is a common practice in the biological evaluation of medicines and is fundamental for the initial analysis of their efficacy. In this study, the cytotoxic effect of Momordica was evaluated on osteosarcoma cell lines, keratinocytes, and mesenchymal stem cells. The results showed that Momordica had pronounced cytotoxicity activity on osteosarcoma cells even at low concentration.

INTRODUCTION

Several plants have been described throughout history with varied biological activities beneficial to re-establish human and animal health. *Momordica charantia* is among these plants and has been popularly used for medicinal purposes for thousands of years. This plant belongs to the Cucurbitaceae family and is popularly known as bitter gourd, balsam pear, bitter melon, Saint Caetano melon, kugua, or karela (Habicht et al., 2011). *Momordica* is widely cultivated in the tropical and subtropical regions of the world, such as some parts of Asia, Africa, Oceania, Central America, and South America (Shan et al., 2012). The whole plant has significant pharmacological effects, especially the seeds and fruits. Various medicinal properties of *M. charantia* have been studied over the years, including the hypoglycemic, antibacterial, antiviral, antitumor, immunomodulatory, antioxidant, antidiabetic, anthelmintic, antimutagenic, antilipolytic, hepatoprotective, anti-inflammatory, and antiulcerogenic activities (Habicht et al., 2011). Various fruit bioactive components of *Momordica* have been described in the literature, such as carbohydrates, proteins, lipids (Ayeni et al., 2015; Najafi & Torki, 2010), flavonoids (Liang-juan & Wei-fen, 2007), triterpenoids (Zhao et al., 2014), saponins (Ma et al., 2014; Murakami et al., 2001), polypeptides (Ahmad et al., 2012), and sterols.

Despite the several pharmacological activities of *Momordica*, adverse effects have been reported in recent years that limit its wider application. In addition to some toxic signs, previous studies have concluded that this plant may induce clinical signs such as hypoglycemic coma in children and miscarriage or even death in laboratory animals (Grover & Yadav, 2004). For these reasons, it is important to evaluate the *in vivo* and *in vitro* efficacy of its therapeutic properties to offer the safest pharmaceutical form, reducing its toxic effects on patients and being effective in its indication.

Within this context, homeopathy, a therapeutic technique used for over 200 years with beneficial effects on health with reduced toxic effects, is becoming increasingly popular in many countries. However, there is still little knowledge about its experimental validation and mechanisms of action. Several doubts exist about using diluted and dynamized medicines (exceeding Avogadro's limit). Consequently, validation through well-designed experiments is needed to prove the safety and efficacy of these medicines (Samadder et al., 2013).

The *in vitro* assessment of cell viability is a common practice in the biological evaluation of products and medicines and is fundamental for the initial analysis of their efficacy. Exposing cultured cells to the substances of interest makes it possible to characterize the effects of cytotoxicity reactions (D. K. Lee et al., 1998). The tests can be performed with several cell lines. Keratinocytes, tumor lines, and mesenchymal stem cells (MSC) are well-established as models to evaluate the safety of products by evaluating cytotoxicity (C. W. Lee et al., 2022; Lin et al., 2022; Nicolas-Espinosa et al., 2022). Therefore, this study aimed to compare the cytotoxic effect of *Momordica* D5 on osteosarcoma cell lines, keratinocytes, and mesenchymal stem cells.

METHODS

Cell Cultivation

This study used mesenchymal stem cells (MSC) derived from adipose tissue, bone tumor lineage – osteosarcoma – (U2OS), and immortalized keratinocytes originating from human skin (HaCat). Per manufacturer recommendations, cells were grown in Dulbecco's Modified Eagle Medium (DMEM - Sigma-Aldrich).

Preparation of homeopathic *Momordica*

The Mother Tincture was the starting point for preparing the tested medicine (*Momordica* D5). The Hahnemannian Decimal Method was used, as described in the Brazilian Homeopathic Pharmacopoeia. One part of the active ingredient was mixed with 9 parts of the inert ingredient, using a sterile isotonic solution, and succussed 100 times, yielding Momordica D1 (1×10^{-1}). Then, 1 part of *Momordica* D1 was used with 9 parts of the inert ingredient and succussed 100 times, yielding *Momordica* D2 (1×10^{-2}). The successive dilution continued until potency D5 was obtained. The medicine was then bottled in 1.1mL ampoules.

Cell Viability

The cytotoxicity of the compound on mesenchymal stem cells, osteosarcoma cells, and keratinocytes was determined by the MTT test. The test is a colorimetric assay that measures the reduction of {[3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium]} (MTT) from cellular mitochondrial activity. For this assay, cells were initially cultured in triplicate in 96-well plates containing 1 x 10^4 cells/mL in culture medium and kept in an incubator at 37.5 °C, 5% CO_2 for 24 hours for stabilization and cell adhesion. After this period, the *in vitro* culture

medium was replaced by the treatment medium containing *Momordica* D5 at concentrations of 10 and 50 µL/mL, and the cells were cultured for additional 48 hours. Subsequently, the culture medium was removed, and 100 µL of the solution containing 3-(4,5-dimethylthiazol-2-yl bromide)-2,5-diphenyl tetrazolium (Sigma-Aldrich No. M2128) at 0.5 mg/mL was added to each well. The plates were incubated for 4 hours at 37.5°C, 5% CO_2, and protected from light. Finally, the supernatant was removed, 100µL of DMSO was added to each well, homogenized, and evaluated in a microplate spectrophotometer measuring the absorbance in the 570 nm spectrum (Molecular Devices, Sunnyvale, CA, USA) to identify the density of viable cells.

Statistical Analysis:

Cell viability was calculated based on the absorbance obtained in the control group. Tukey's multiple comparison test analyzed the percentages obtained using the GraphPad Prism® 7.04.

RESULTS AND DISCUSSION

This study evaluated the difference in cytotoxicity of the 10 and 50 µL/mL doses of *Momordica* D5 in mesenchymal stem cells (MSC) from adipose tissue, osteosarcoma (U2OS), and keratinocytes (HaCat). The results show that even at a lower concentration (10 µL/mL), *Momordica* D5 showed cytotoxic action for the tumor cell lineage and did not significantly affect the viability of MSC and HaCaT (Figure 1 A). Increasing the dosage to 50 µL/mL increased the cytotoxic effect of the medicine on tumor cells, practically eliminating their viability (Figure 1 B).

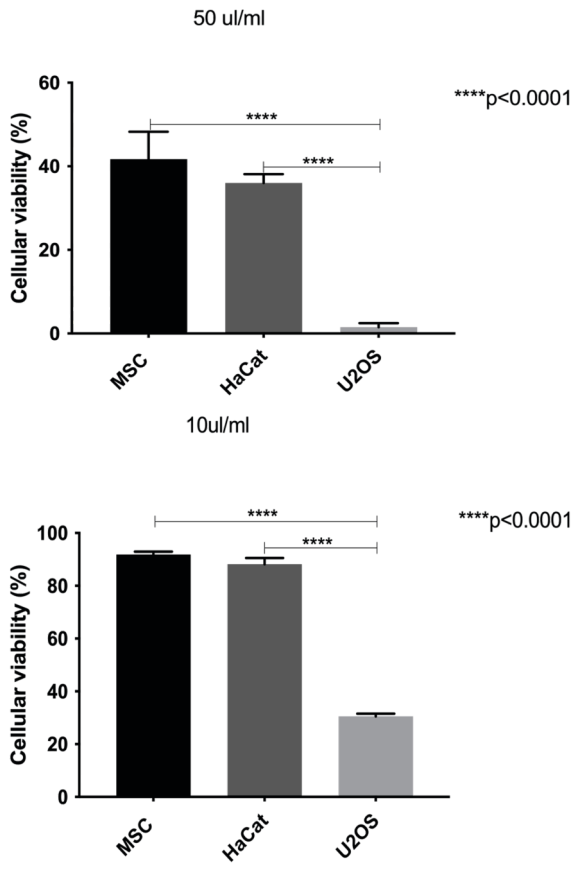

Fig 1: Cell viability of mesenchymal stem cells (MSC), keratinocytes (HaCat), and osteosarcoma cells (U2OS) after 48h of treatment with *Momordica* D5 at 10 µL/mL (A) and 50 µL/mL (B).

Although this study is a pioneer in testing *Momordica* in homeopathic dilutions in osteosarcoma cells, previous studies have already shown that *Momordica* extracts (herbal medicine) presented growth inhibition effects on murine colon tumor cells, choriocarcinoma, melanoma, breast cancer, skin tumor, prostate cancer, squamous carcinoma of the tongue and larynx, and Hodgkin disease (Basch et al., 2003; Grover & Yadav, 2004; Habicht et al., 2011; Licastro et al., 1980). Tests with proteins purified from *M. charantia* seed demonstrated anticancer activity. They inhibited protein and, subsequently, DNA synthesis in normal (stimulated by mitogens) and leukemic lymphocytes in human peripheral blood (Licastro et al., 1980). In 1996, Battelli and collaborators used *Momordica* proteins linked to the monoclonal antibody 48-127 (MAb), and they were able to recognize a glycoprotein (gp54) expressed in human bladder tumors and human bladder carcinoma cell lines. Under ideal conditions, the *M. charantia* preparations were able to reduce cell multiplication, and no toxicity was observed (Giulia BATTELL et al., 1996). The anticarcinogenic effect of the fruit aqueous extract of *M. charantia* was also studied in a model of cutaneous carcinogenesis in mice. The study verified that the oral administration of the fruit extract had an adverse effect on the animals' overall health and life expectancy when used in high concentration (Basch et al., 2003)

As described for other cancer cell lines, even in homeopathic dilution, *Momordica* showed a high ability to inhibit the growth of bone tumor cells, presenting low inhibition of healthy cells. Therefore, these findings emphasize its selective effect on tumor cells when tested *in vitro*, diluted, and dynamized.

CONCLUSION

This study showed that even at low concentration *Momordica* D5 has cytotoxic action on osteosarcoma cells and little cytotoxic effect on healthy cells (mesenchymal stem cells and keratinocytes), thus demonstrating this medicine's *in vitro* action potential and selectivity/predilection/affinity for tumor cells compared to MSC and HCat. Therefore, the use of this homeopathic medicine as an adjunct to cancer treatment may be further evaluated, opening possibilities for additional *in vivo and in vitro* tests of this medicine.

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