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RESEARCH ARTICLE

Comparison of homeopathic medicines composed of Taraxacum officinale and Lycopodium clavatum for treating cellular hepatocarcinoma

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ABSTRACT

Homeopathy has been increasingly standing out for its proven clinical benefits and for providing a course of treatment with fewer side effects to patients, thus promoting a better quality of life for them. Homeopathic therapy can be used in different contexts and is an additional alternative for treating cancer patients in Integrative Medicine. Within this context, hepatocellular carcinoma has a high mortality rate and, in most cases, is diagnosed in advanced cases. Therefore, this study evaluated the in vitro efficacy of the association of the homeopathic medicines Taraxacum officinale and Lycopodium clavatum against HepG2 cells. For this purpose, cells were grown in 75 cm² flasks and then plated in 96-well plates. The treatment with Taraxacum D4 + Taraxacum D8 + Taraxacum D12 and Taraxacum D5 + Lycopodium D6 at concentrations of 20, 40, and 60 µL/mL was added to each well. After 48 hours of incubation in an oven at 37°C, 5% CO2, cells were subjected to the cell viability test by MTT. A decrease in viability was observed compared to the control group (cells without treatment). In addition, the medicine Taraxacum officinale was more effective in decreasing cell viability than the other medicines in the three conditions tested. In conclusion, the homeopathic medicine Taraxacum D4 + Taraxacum D8 + Taraxacum D12, in accord of potencies, is promising against hepatocellular carcinoma cells and can be used to aid in the treatment of this type of cancer.

Introducution

Homeopathy is a therapy of German origin known to use highly diluted animal, vegetal, and mineral medicines to treat a variety of diseases, being able to provide improvements to patients without presenting side effects, especially in cases of chronic diseases. The search for less invasive therapies for health care with good results and no adverse effects is growing[1,2] .

Among the various medicines that compose the homeopathic medical matter, Taraxacum officinale and Lycopodium clavatum stand out for having important activity in the liver environment. Taraxacum officinale, popularly known as "dandelion", is a plant of European origin that has been used for medicinal purposes since ancient times[3]. The main phytochemicals found in this plant are carotenoids, flavonoids (quercetin, chrysoeriol, luteolin-7-glucoside), phenolic acids (caffeic acid, chlorogenic acid, chicoric acid), polysaccharides (inulin), sesquiterpene lactones (taraxinic acid, taraxacoside, 11β ,13-dihydrolactucin, ixerin D, taraxacolide-O-β-glycopyranoside), sterols (taraxasterol, β-sitosterol, stigmasterol), and triterpenes (α-amyrin)[4]. Among these compounds, taraxasterol has been studied for its antitumor activity, capable of causing cancer cell death and inducing nitric oxide production[5].

Taraxacum officinale has hepatoprotective activity, and several studies are being carried out to prove and evaluate its beneficial effects on patients under treatment for liver diseases. In addition, the plant has antifungal, antiviral, and antibacterial activities. It also stimulates the immune system's release of cytokines and nitric oxide[6], and it has been indicated for hepatopathies such as jaundice within the Homeopathic Medical Matter[7].

Lycopodium clavatum is another medicine known for its beneficial effects against tumors, inflammatory diseases, and neurological diseases (Alzheimer's), among others[8]. In vitro tests performed with homeopathically-potentized ultra-high dilutions of L. clavatum proved its antitumor activity against HeLa cells (cervical cancer) due to the cytotoxic effect after contact of the cells with the medicine[9]. It is also indicated by the Homeopathic Medical Matter for treating hepatopathies and kidney diseases[7].

Although homeopathic medicines are promising and present benefits to the patient's life, it is important to study which medications have the most benefits for each type of disease. Therefore, in vitro tests are paramount to ensure the understanding and optimization of the use of such medicines. This study evaluated the effect of two homeopathic medicines on HepG2 cells. The first medicine was composed of different dilutions of Taraxacum officinale, and the second medicine was composed of Taraxacum officinale and Lycopodium clavatum.

Method

The Mother Tincture was used as the starting point to prepare the tested substance (Taraxacum D4, D5, D8, and D12; Lycopodium D6). 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 Taraxacum D1 (1×10^{-1}). Then, 1 part of Taraxacum D1 was used with 9 parts of the inert ingredient and succussed 100 times, yielding Taraxacum D2 ($\times10^{-2}$). The successive dilution continued till D4, D5, D8, and D12 were obtained. The same

procedure was done for Lycopodium dilutions. This product was then bottled in 1.1 mL ampoules. After preparing each potency separately, the medicines are combined using equal parts of each one.

CELL CULTURE

HepG2 cells were obtained from a commercial bank and grown in 75 cm² culture flasks with Dulbecco's Modified Eagle Medium (DMEM) supplemented with an antibiotic and fetal bovine serum. The culture flasks were incubated in an oven at 37 °C, 5% CO2, and the culture medium was changed every 48 hours until the cells reached a confluence between 60-80%.

Subsequently, these cells were trypsinized and plated in 96-well plates at 10,000 cells per well. After 24 hours of incubation under the same conditions described above, these cells were treated using homeopathic Taraxacum D4 + Taraxacum D8 + Taraxacum D12 and Taraxacum D5 + Lycopodium D6 at concentrations of 20, 40, and 60 µL/mL. The control group was not submitted to treatment. The plates were incubated for another 48h in an oven. After incubation, the treatment medium was removed from the wells, and the MTT reagent was added. Then, the plate was placed back in the oven at 37 °C, 5% CO2, for 4 hours. After this period, dimethyl sulfoxide (DMSO) was added to the wells, and absorbance was read in a spectrophotometer. The results were tabulated, and cell viability (%) was quantified relative to the control treatment.

STATISTICAL ANALYSIS

Absorbance analysis results obtained in the spectrophotometer were tabulated, and cell viability (%) was calculated compared to the control group. These data were submitted to statistical analysis by Student's T test in Prisma Version 9.5.1 (528).

Results

CELL VIABILITY

Cell viability of HepG2 cells after treatment with two homeopathic medicines at 20, 40, and 60 µL/mL was calculated based on the control group (which received no treatment). Tables 1 and 2 show the values obtained for cell viability in each case. Statistical analyses were performed using quadruplicates for each concentration.

The association of Taraxacum officinale with Lycopodium clavatum resulted in higher viability of tumor cells compared to the medicine composed solely by potencies of Taraxacum.

The viability values observed in the treatment with Taraxacum officinale were 42.75, 16.25, and 9.25% for 20, 40, and 60 µL/mL, respectively. In contrast, the treatment with Taraxacum officinale and Lycopodium clavatum showed viabilities of 66.75, 49.5, and 33% for 20, 40, and 60 µL/mL, respectively. Therefore, Taraxacum officinale was more harmful to tumor cells when used solely than associated with Lycopodium clavatum

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Table 1. Cell viability of HepG2 cells after treatment with Taraxacum D4 + Taraxacum D8 + Taraxacum D12 for 48 hours.

Taraxacum D4 + Taraxacum D8 + Taraxacum D12			
Cell viability (%)			
	20 µL/mL	40 µL/mL	60 µL/mL
v1	44	18	10
v2	40	17	9
v3	42	17	9
v4	45	13	9
Mean	42.75	16.25	9.25

v: cell viability

Table 2. Cell viability of HepG2 cells after treatment with Taraxacum D5 + Lycopodium D6.

Taraxacum D5 + Lycopodium D6			
Cell viability (%)			
	20 µL/mL	40 µL/mL	60 µL/mL
v1	63	47	36
v2	65	50	32
v3	63	49	34
v4	76	52	30
Mean	66.75	49.5	33

v: cell viability

The data obtained were compared using Student's T-test. The following figure compares the results, confirming that using *Taraxacum officinale* exclusively was more effective since the association with *Lycopodium clavatum* caused less tumor cell death in the three conditions evaluated.

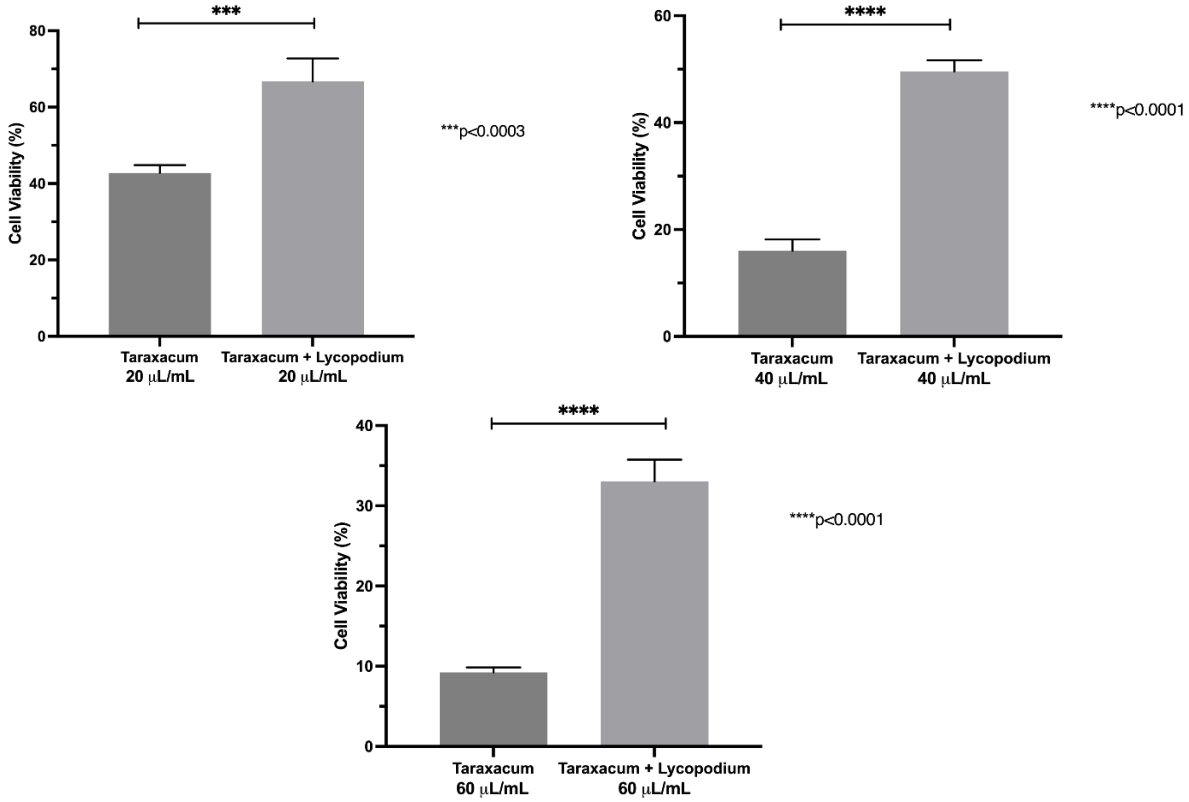

Figure 1. Comparison of treatments performed on HepG2 cells with Taraxacum D4 + Taraxacum D8 + Taraxacum D12 and Taraxacum D5 + Lycopodium D6 at 20 µL/mL, 40, µL/mL, and 60 µL/mL.

Discussion

The reported results proved that the homeopathic medicines evaluated showed antitumor activity in HepG2 cells. These cells come from hepatocellular carcinoma, a type of cancer with a high mortality rate since it is mainly diagnosed in advanced stages[10]. The tests performed in this report showed that treating HepG2 cells with the homeopathic medicine *Taraxacum officinale* resulted in higher cytotoxicity than the treatment combining *Taraxacum officinale* with *Lycopodium clavatum.*

The mechanisms by which *Taraxacum officinale* acts on tumor cells are investigated through diverse approaches, as several compounds are present in this plant. *In vivo* studies assessed the effect of the *Taraxacum officinale* extract on rats with breast cancer, revealing a decrease in the levels of the cancer antigen 15-3 (CA15-3). In addition, the plant extract regulated the PI3K (phosphatidylinositol 3-kinase) and Akt (protein kinase B) pathways, which are involved in suppressing breast cancer growth and proliferation[11].

The mechanism of action of *Taraxacum officinale* was also evaluated in neuroblastoma cells. This investigation revealed apoptosis and disruption of mitochondrial integrity, accompanied by the inhibition of invasion and migration in tumor cells exposed to the plant extract. The effect of this plant in simultaneous treatment with the *Viscum album* extract was also evaluated, and the results pointed to a synergistic effect between the compounds[12]. In the current study, resulting from the combination of two compounds was not observed since the cytotoxic activity was more

pronounced when *Taraxacum officinale* was used in isolation, as opposed to its co-administration with *Lycopodium clavatum*.

According to Yamada et al. (2020), quercetin and myricetin suppress HGF- and TGF-αgrowth factors in HepG2 cells. These growth factors play a crucial role in the metastasis and recurrence of hepatocellular carcinoma[13]. The *Taraxacum officinale* extract was also reported to cause alterations in the genetic pathways associated with tumor cell proliferation and survival, resulting in cell apoptosis in an *in vivo* study performed in rats under treatment for breast cancer. Induction of apoptosis is a target in cancer treatments because it causes suppression of tumor progression[14].

Ren et al. (2022) evaluated the in vitro effect of the medicine taraxasterol extracted from *Taraxacum officinale*. The authors noted that the medicine inhibited proliferation and caused apoptosis and cell cycle arrest in HepG2 and Huh-7[15]. Our results corroborate these authors regarding the proliferation inhibition effect, culminating in high toxicity. However, our study used the plant in its homeopathic scale, which reinforces and proves the effectiveness of homeopathic medicines in controlling diseases, in this case, represented by hepatocellular carcinoma cells.

In addition to the action of *Taraxacum officinale*, studies have also proven the effectiveness of using *Lycopodium clavatum* for cancer treatment. In an *in vivo* study performed in mice with liver cancer, the homeopathic medicine *Lycopodium clavatum* (Lyco-200) was used as a treatment, showing its effectiveness in decreasing cell viability, inducing apoptosis, and reducing cytogenetic damage[16]. Giang et al. (2022) also evaluated different compounds obtained from *Lycopodium clavatum* and proved their action in HepG2 cells[17]. The effect of *Lycopodium clavatum* was also evaluated using homeopathic potencies (LC-5C and LC-15C). An anticancer effect against cervical cancer cells (HeLa) was observed, characterized by the induction of apoptosis[18]. Therefore, its influence might be more pronounced when employed individually, as observed with *Taraxacum officinale*.

The combination of *Taraxacum officinale* and *Lycopodium clavatum* tested in this study corroborates the abovementioned studies. However, *Taraxacum officinale* used in accord of potencies, showed higher cytotoxic activity in HepG2 cells *in vitro*, resulting in a greater decrease in cell viability. Therefore, it had a more pronounced effect on cancer cells than the homeopathic medicine composed of *Taraxacum officinale and Lycopodium clavatum*. Further tests must be performed using *Lycopodium clavatum* to prove its solo action on HepG2 cells.

Conclusion

This work provides evidence of the action of homeopathic medicines composed of *Taraxacum officinale* and *Lycopodium clavatum* in hepatocellular carcinoma (HepG2) cells. Although the two medicines decreased the viability of tumor cells, *Taraxacum*, in accord of potencies, was more efficient than the association of *Taraxacum* + *Lycopodium*. Therefore, homeopathy may aid in treating patients diagnosed with Hepatocellular Carcinoma, as it provides good results, probably increasing the life expectancy of these patients.

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