Studying the Effect of Curcumin on Hep3B Hepatocarcinoma in Molecular and Biology Research Laboratory at An-Najah National University

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Research article

Keywords: Curcumin, Hep3B cells, Hepatocellular carcinoma, Proliferation, Apoptosis Necrosis, Cytotoxicity

Posted Date: July 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-708327/v1>

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Abstract

Liver cancer is the growth and spread of cells in the liver that are abnormal. In this research, we examined Curcumin's effects on the growth of Hep3B cells liver in Molecular and Biology Research Laboratory at An-Najah National University. Curcumin regarding several theoretical and practical studies starves cancer cells to death. The life cycle of Hep3B cell has been analyzed by MTS assay, which is used to assess cell proliferation, cell viability, cytotoxicity, and flow cytometry. Our results agreed that Curcumin could a potentially efficient medication in the treatment of hepatocellular carcinoma, according to the findings of this study which confirms that curcumin treatment inhibited the growth of Hep3B. Thus, taking curcumin in our food is recommended.

Introduction

How can curcumin effect our health? What are the aspirations towards curcumin and its relationship to cancer cells and their growth as it is starving abnormal cells to death? And can we reduce the spread of liver cancer in particular?

Scientists worldwide have drawn attention to Curcumin (diferuloylmethane), an orange-yellow component of turmeric or curry powder [1] for its biological activities. (e.g., antioxidant, anti-inflammatory, antimicrobial, antiviral). Among them, its anti-cancer ability has been the most mentioned and is still under investigation. This polyphenol molecule, alone or in combination with other medicines, could be a promising cancer treatment, curcumin therapy inhibited Hep3B growth, induced cell apoptosis, as well as reduced necrosis [2]. Humans have been utilizing plant sources either net shape or crude extracts to treat numerous illnesses. The most concentrated research on herbs with immune-stimulating properties, it is a good advantage to reduce the risk of cancer [3].

Curcumin is a key component of the Curcuma species, which is often utilized in food as a yellow coloring and flavoring element [4]. also considered the first drink in India (golden milk), it helps to promote healthy and beautiful skin, strengthen hair and treat its problems and It plays an important role in preventing or slowing the progression of Alzheimer's disease by removing plaques resulting from the accumulation of beta-amyloid protein among the brain cells that cause Alzheimer's [5]. Another important effect of Curcumin is the modification of the immune system, which neutralizes the development of cancer. This suggests the need to develop it as a chemotherapy agent [6]. The molecular system of the hepato-protective activity of Curcumin is because of its cancer prevention agent properties and inhibitory actions against nuclear factor (NF)- κ B that adjust various pro-inflammatory and profibrotic cytokines [7].

Overall, scientific evidence suggests that curcumin has a strong curative potential for liver diseases. Curcumin's effects on cell development and survival factor expression may give a benefit in the therapy of cancer progression without the direct radio sensitizing influence of curcumin on these cells [8]. Primary liver cancer, otherwise called hepatocellular carcinoma (HCC), is a standout amongst the deadliest diseases having overall spread [9]. is linked to persistent inflammation and fibrosis caused by a variety of

causes, such as hepatitis B (HBV) and C (HCV), alcohol consumption, and fatty liver diseases [10]. In conclusion, Hepatoma cell survival is negatively affected by Curcumin therapy where Curcumin starves cancer cells to death.

In our research, we aimed to study the Effect of Curcumin on Hep3B Hepatocarcinoma in Molecular and Biology Research Laboratory at An-Najah National University.

Materials And Methods

This research was done in Molecular and Biology Research Laboratory (G0021) at An-Najah National University using the Roswell Park Memorial Institute (RPMI) which is a culture media for hepatoma Hep3B [11]. RPMI is supplemented with 1% Antibiotic penicillin-streptomycin for routine culture which affects bacterial inhibition [12], 1% Glutamine for tumor cell proliferation [13], and 10% fetal bovine serum (FBS) which most often used in cell culture media as a supplement [14], because the serum is an ill-defined component in cell culture medium in general [15]. MTS(3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) kit was used to determine the anti-proliferative effect of Curcumin poly-phenol by assessment of cell viability, cytotoxicity, and proliferation [16].

2.1 Cell Culture

Hep3b hepatoma cells in this study were taken from an eight-year-old child with Hepatocellular carcinoma (HCC). The cells were grown in RPMI with Dimethyl sulfoxide (DMSO) used for freezing cells [17], which prohibit the formation of ice crystals during the icing process, otherwise, cells would be destroyed, then these cells were saved at -150°C . 2 flasks of 75cm have been used to seed the cells after removing of old media which filled with waste. Then washed with Phosphate Buffer Saline (PBS), cells were digested with Trypsin for allowing a cell to detachment [18], and RPMI media were added for nutrition [19]. Finally, the cells were counted by chamber to find the volume of fluid from the cells to be cultured later. The values were calculated using the following equation:-

$$M1 \times V1 = M2 \times V2$$

M 1: concentration of cells, which cells had been counted by chamber

M 2: standard concentration of cells depends on MTS.

V 1: volume of cells.

V 2: Final volume of media.

2.2 MTS assay for cell viability determination

2138 μl of RPMI were mixed with 262 μl of cells then 200 μl of the mixture were taken and seeded into 12-well tissue culture plate then incubated for 24 hr. at 37°C with presence of 5% CO_2 to allow cells to adhere. 24 hours later, cell growth was examined through the microscope to ensure its growth, then were treated

with 0,5,10,15,20,40 mg/ml Curcumin at at **37°C** (98.6°F) for 2 hours. The activity of the cells was then determined by adding into each well 20 µl of MTS and 100 µl RPMI and incubating at 37°C for 2 hrs. A Microplate Reader was used to determine the absorbance at 490 nm. Keep in mind that when spectrophotometers was compared with microplate, there was no statistically significant difference between the spectrophotometer in single wavelength mode and the microplate reader in dual-wavelength mode [20].

2.3 Apoptosis detection

The Hep3B were seeded into a 12-well tissue culture plate and treated with different concentrations of Curcumin 0, 5, 10, 15, 20, 40 mg/ml then incubated at 37°C for 24 hr. The apoptosis of the cells was then observed using flow cytometry and a cell apoptosis detection package. The cells were taken, washed two times in PBS, After that, the cells were incubated in 500 µl of binding buffer. The cells were then treated for an additional 15 minutes with 5 µl Annexin V- fluorescein isothiocyanate and 5 µl propidium iodide (PI). Flow cytometry was used to investigate cell apoptosis.

2.4 Necrosis Detection

There are two types of cytotoxicity assays for assessing necrosis.: those based on the differential absorption of DNA-binding dyes that do not cross the plasma membrane of living cells, such as propidium iodide, and those based on intra cellular molecule leakage via a compromised plasma membrane. When the plasma membrane is destroyed, lactate dehydrogenase (LDH), the extracellular area is flooded with a soluble cytoplasmic enzyme prevalent in practically all cells [21]. This assay uses a tetrazolium salt to detect LDH leakage into the cell culture medium.

Results

4.1 Curcumin inhibits the proliferation of Hep3B

After isolation, Hep3B was treated with, 0–40 mg/ µl Curcumin and MTS was used to detect the spread of Hep3B, Hep3B prevalence decreased significantly after treatment with 5,10,15, 20, and 40 mg/ml of Curcumin compared to the untreated control well, as shown in Fig. 1. These findings revealed that Curcumin administration greatly reduces Hep3B proliferation, with higher Curcumin concentrations resulting in even more reduction.

4.2 Curcumin induces the apoptosis of Hep3B.

After Curcumin administration, flow cytometry was performed to identify cell death. When compared to the untreated control group, hep3B apoptosis increased considerably. These findings revealed that Curcumin administration caused Hep3B to apoptosis, as seen in Fig. 2.

4.3 Curcumin reduces the Necrosis of Hep3B

Hep3B necrosis was reduced as compared to the untreated control group. Figure 3 showed That Curcumin therapy reduced the necrosis of Hep3B.

Discussion

hepatocellular carcinoma is a type of cancer that has a multi serious influence on people's health. Curcumin's effects on Hep3B growth were investigated in in Molecular and Biology Research Laboratory (G0021) at An-Najah National University – 2019–2020. According to the findings, curcumin inhibited replication, promoted apoptosis, and reduced necrosis in Hep3B cells.

According to current research, curcumin may have a powerful anti-cancer effect. Curcumin has also been demonstrated in previous studies to have anti-cancer properties, reducing cancer-related proliferation, migration, and angiogenesis in a range of cancer types [22 & 23], including several cancers of the breast [24], lung [25], colon [26], renal [27], ovarian carcinoma (silent killer) [28], pancreatic [29], osteosarcoma (also called osteogenic sarcoma) [30], T-cell acute lymphoblastic leukemia (T-ALL) [31], and the liver [32].

Targeting liver cancer stem cells is critical to avoid tumor recurrence since cancer cells show the capacity to self-restore, distinguish the difference, in addition start and prolong tumor growth. Curcumin was discovered in the current study to suppress Hep3B development. Curcumin has been shown to suppress glioblastoma stem cells from proliferating in the past, which is consistent with current findings [33].

Other research has found that contemporary malignant growth treatments based on reprogramming techniques can be applied even in tumor cells with a complex, unresolved genetic state. Hep3B cells were more effective in reprogramming than the other liver cancer cell lines. The properties of the reprogrammed Hep3B cells were similar to those of pluripotent stem cells [34].

In the twenty-first century, cancer will be the leading cause of death, and natural products should deliver innovative and more potent anticancer drugs [35]. Several clinically useful anti-cancer medicines have been created from natural compounds generated from plants. The number of promising novel drugs under clinical development based on selective activity against cancer-related molecular targets demonstrates that plants continue to play an important role in drug discovery [36]. Alkaloids (berberine), flavonoids (gambogic acid, silibinin, and wogonin), terpenes (artemisinin, ursolic acid, triptolide, and oridonin), quinones (emodin and shikonin), and saponins (ginsenoside Rg3) are among the natural compounds with anticancer potential [37].

Limitation

Difficulty in analyzing the results of curcumin effect on the cells due to the lack of apoptosis and necrosis tests in the laboratory environment of the university, which forced to send samples to other laboratories for analysis.

Recommendations

As curcumin helps to promote health in many aspects as well as reduces Hep3B necrosis, induces the apoptosis of Hep3B, and inhibits the proliferation of Hep3B, its uses are recommended to be as a part of our nutrient culture.

Declarations

Conflicts of Interest

The authors declared that they have no competing of interests in this manuscript's publication.

Acknowledgements

The authors are kindly acknowledging Dr. Iyad Al Ali the Scientific Research Coordinator, Dr Jonny Amer the Research Coordinator, Liver Unit, Hadassah University Hospital, Ein Kerem-Jerusalem, Mr. Ahmad Mosa the supervisor of (G0021) laboratory at Faculty of Medicine and Health Sciences, A-Najah National University for facilitating the investigation of Curcumin's effects on Hep3B growth in Molecular and Biology Research Laboratory (G0021) at An-Najah National University, 2019-2020.

Authors' Contributions

Dr Wafaa Menawi supervised the action planning and manuscript writing and finalization, Aseel Raddad conceived, designed and performed the experiments, Reem Abu Baker and Haya Shurafa wrote the paper. All of the authors have read and approved the final version of the paper.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was conducted as a graduation project for a BSc graduate studying in the Faculty of Medicine and Health Sciences at An-Najah National University. It received no funding from any source.

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Figures

Figure 1

Curcumin inhibited the proliferation of Hep3B.

Figure 2

Curcumin-induced apoptosis in Hep3B cells.

Figure 3

Curcumin suppresses Hep3B necrosis.