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RESEARCH ARTICLE
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EVALUATION OF CYTOTOXIC ACTIVITY OF CANAGLIFLOZIN IN HEPG2 CELL LINES

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ABSTRACT
This study examines the cytotoxic effects of Canagliflozin on HepG2 cell lines, serving as models for hepatocellular carcinoma (HCC). Canagliflozin was purchased in powder form from CCL pharmaceuticals. HepG2 cells were cultured in DMEM medium and plated in 96-well plates, where Canagliflozin was administered at various concentrations. Cell viability was assessed using crystal violet staining, and the half-maximal inhibitory concentration (IC_{50}) was determined computationally. Results showed moderate cytotoxicity of Canagliflozin, with an IC_{50} value of 111 µM. The study highlights the potential of Canagliflozin as an anticancer agent and the need for further research to understand its mechanism and optimize therapeutic potential. Overall, this study contributes to the growing evidence supporting the use of Canagliflozin for cancer treatment.

Keywords: Cytotoxicity, Canagliflozin, hepatocellular carcinoma, IC_{50}

Introduction
Canagliflozin is a sodium glucose transporter (SGLT) inhibitor that was extracted from an apple tree's bark(1). It is commonly used for the treatment of type 2 diabetes mellitus by inhibiting SGLT2 in the kidneys, facilitating glucosuria(2)(3). In addition to its antidiabetic effect, Canagliflozin has shown cytotoxic acitivity in colon, hepatic, pancreatic, prostatic and breast cancer cells(4). As cancer cells are dependant on glucose for their survival, inhibition of glucose uptake by cancerous cells could potentially be the mechanism of the cytotoxic effects of Canaglifllozin on these cells. However, it has been noted that Canagliflozin also inhibits Complex I of the respiratory chain in the mitochondira in mouse hepatocytes and human embryonic kidney cells by activating AMP- activated proyein kinase(5).

Over the past two decades, hepatocellular carcinoma (HCC), the most common primary liver cancer, has emerged as the fastest-growing cancer among adults and ranks as the third leading cause of cancer-related mortality worldwide(6)(7). Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), leading to liver inflammation, fibrosis, and cirrhosis, are the major contributors to the

development of HCC(8). In Pakistan, the burden of HBV and HCV infections is significant, with a prevalence rate of 2.4% and 3.0%, respectively which is among the highest worldwide(9). The management of early-stage HCC primarily relies on surgical resection and transplantation because the efficacy of medical options is limited. Therefore, there is a critical need for the development of novel therapeutic approaches and the optimization of existing treatments to improve outcomes for patients with HCC.

This study aims to explore the potential of Canagliflozin as an anticancer agent, specifically targeting hepatocellular carcinoma. We used Sorafenib as a control drug which has been approved by the FDA in 2007 for advanced second and third stages of HCC. Sorafenib is a multikinase inhibitor that mainly acts on VEGFR2, PDGFR and KIT(10).
By investigating the cytotoxic effect of Canagliflozin on HepG2 cell lines, we hope to contribute to the growing body of evidence supporting the use of natural compounds in cancer treatment. Understanding the mechanisms and optimizing the therapeutic potential of these natural compounds could lead to more effective and less toxic cancer therapies in the future.

Methods

Chemicals and Reagents

- **Canagliflozin powder:** Purchased directly from CCL pharmaceuticals
- **DMEM (Dulbecco's Modified Eagle Medium):** Used as the cell culture medium
- **Fetal Bovine Serum (FBS):** Supplement for DMEM medium (10%)
- **Antibiotics:** Penicillin and streptomycin (2%)
- **Crystal violet stain:** For cell viability assay and cytotoxicity determination
- **Reagents for serial dilution:** For preparing different concentrations of Canagliflozin

Cell Culture: HepG2 cells were acquired from the Cell Culture Precision Oncology Laboratory, Khyber Medical University Peshawar. The cells were revived in T25 flasks and cultured in DMEM medium supplemented with 10% FBS and 2% antibiotic (penicillin and streptomycin). The cells were maintained at 37°C in a humidified incubator with 5% CO2 for 24 hours.

Cell Plating: Canagliflozin was dissolved in dimethy sulfoxide (DMSO) at a concentration of 3mg/ml. HepG2 cells were plated in 96-well plate and treated with stock solution in triplicate and incubated for 48 hours. Different concentrations of the Canagiflozin were prepared in serial dilutions ranging from 0 μM, 6.25 μM, 12.5 μM, 25 μM, 50μM, 100 μM, 200 μM, and 400 μM. These dilutions were added to the cells in the 96-well plates, which were then incubated for another 48 hours.

Cytotoxicity Assay: Cell viability was assessed using crystal violet staining. The staining method involved fixing the cells with formaldehyde, staining with crystal violet, washing, and then solubilizing the dye in acetic acid. The absorbance was measured at 630 nm.

IC50 Determination: The half-maximal inhibitory concentration (IC_{50}) was determined using a dose-response curve, calculated from the concentration at which 50% of cell inhibition was observed. The IC_{50} value was derived from the dose-response curve using the analytical equation ($y = mx + n$).

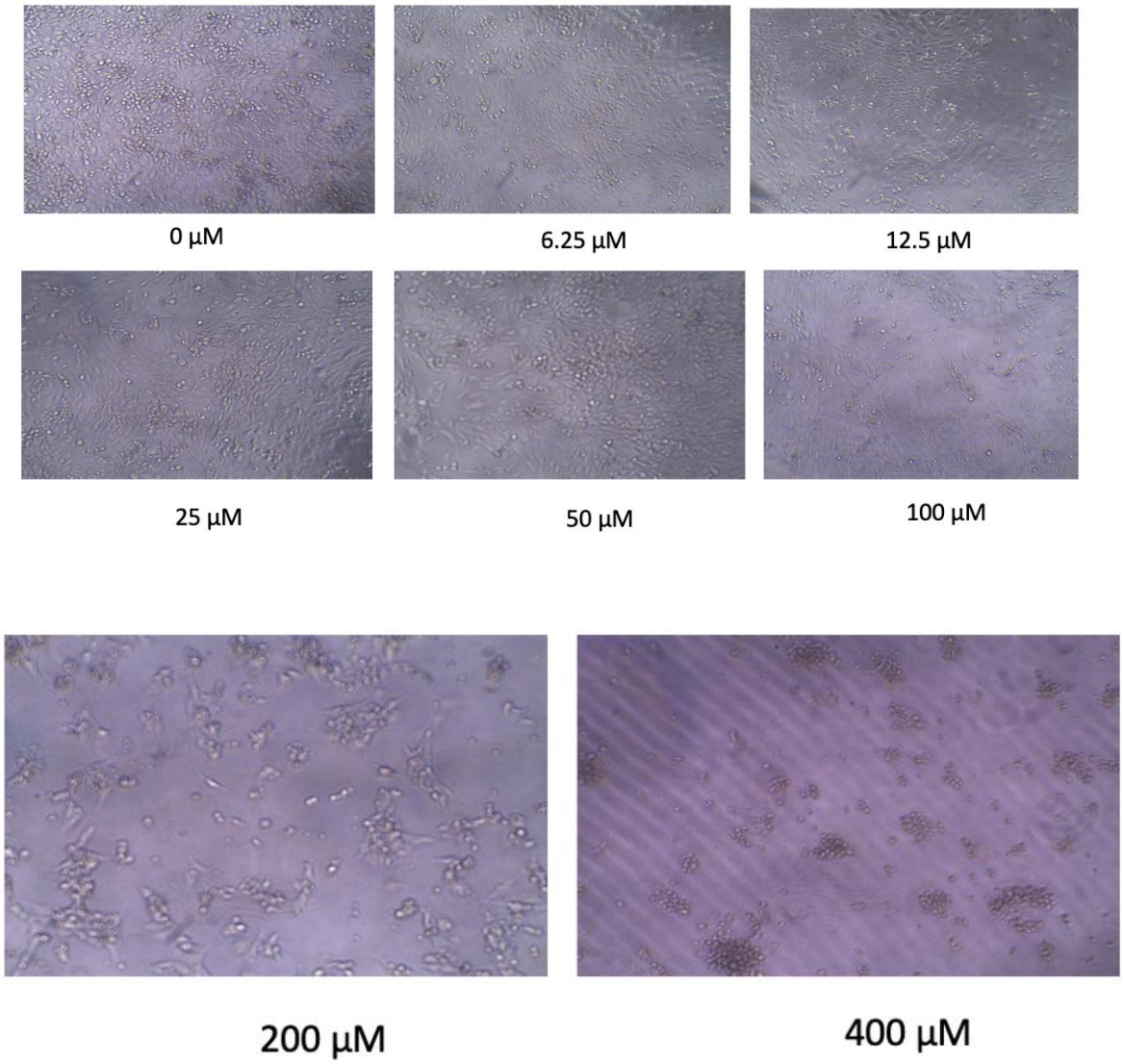
Results

HepG2 cells plated in 96-well plates were used to determine IC_{50} values at 630 nanometers. The experimental procedure included treating the HepG2 cells with varying concentrations of Canagliflozin. The cell viability was assessed using crystal violet staining allowing the measurement of viable cells. The absorbance of the stained cells was measured at 630 nm using ELISA reader.
To determine the half-maximal inhibitory concentration (IC_{50}), a dose-response curve was constructed. Canagliflozin was tested at a range of concentrations, and the effect on the cells was

measured. The percentage of cell viability was calculated and plotted against the concentration of Canagliflozin.

The IC50 value represents the concentration of Canagliflozin required to inhibit 50% of the cell viability. This value was determined computationally using the analytical equation ($y = mx + n$), where 'y' represents the percentage of cell inhibition, 'm' is the slope of the curve, 'x' is the concentration of Canagliflozin, and 'n' is the y-intercept.

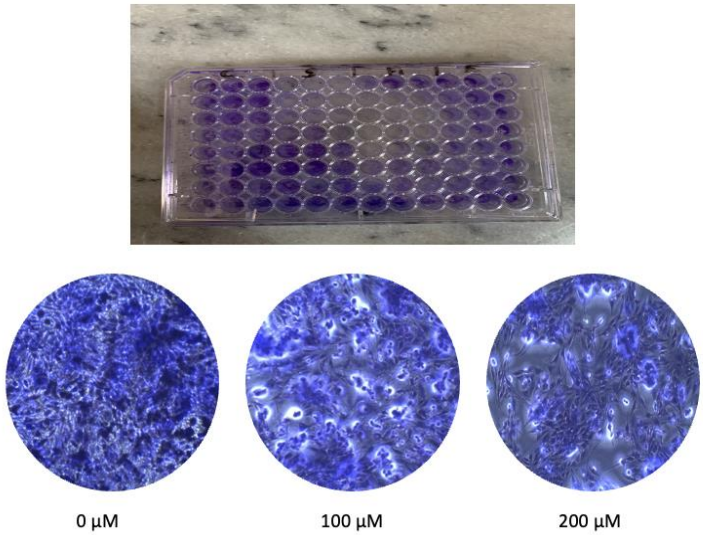
Figure 1: Microscopic images of HepG2 cells (magnification = ×100) following treatment with Canagliflozin after 48 hours of incubation.



The confluency of HepG2 cells progressively decreased, accompanied by a loss of cell-to-cell adherence across canagliflozin concentrations from 12.5 µM to 400 µM. Cell clustering was observed at several points, including 100 µM, 200 µM, and 400 µM. Cell cluster formation became particularly prominent at concentrations of 100 µM, 200 µM, and 400 µM.

The results demonstrated that Canagliflozin exhibited moderate cytotoxicity, with an IC50 value of 111 µM. This indicates that a concentration of 111 µM of Canagliflozin was required to reduce the viability of HepG2 cells by 50%.

Figure 2: **Stained images of HepG cells treated with Canagliflozin at 0 µM, 100 µM and 200 µM**



Discussion

In a study conducted by *Duiyue et al*, the MTT assay was utilized to investigate the effects of canagliflozin on human pancreatic cancer cell lines Capan-1 and PANC-1. The IC50 values for Capan-1 and PANC-1 cells were reported to be 77 µM and 68 µM, respectively. Additionally, treatment with 60 µM canagliflozin showed a similar inhibition of colony formation in cancer cells as the positive control drug, gemcitabine. These findings indicate that canagliflozin suppresses both the growth and colony formation of pancreatic cancer cells(11). *Olga-Demetra Biziotis et al*, studied how canagliflozin affects human non-small cell lung cancer (NSCLC). They tested the drug on several lung cancer cell lines, including A549, H1299, H1975, and H460. Canagliflozin was found to reduce the growth of these cells in a dose-dependent manner. The A549 cells were the most sensitive to canagliflozin, while the H460 cells showed the least sensitivity. The results suggest that canagliflozin, along with radiation therapy, can effectively slow down the growth of NSCLC cells(12). In a study by *Dan Nakano et al.,* SGLT2 expression was confirmed in eight hepatoma cell lines (Huh7, HLF, HepG2, Hep3B, KYN2, KMCH1, HAK1A, and HAK1B) using western blotting. The study demonstarted that proliferation was inhibited in Hep3B and Huh7 cells, with a significant reduction in cell numbers in the 10 µM and 30 µM canagliflozin concentrations(13). Our study results also align with above mentioned research work and indicate that canagliflozin has a dose-dependent effect on cell growth inhibition in HepG2 cell lines. Our findings observed moderate cytotoxicity at concentrations of 100 and 200 micromoles with an IC_{50} value of 111 µM.

Conclusion

Our study contributes to the growing evidence regarding the potential anticancer properties of canagliflozin and suggests that higher doses of canagliflozin are more effective at inhibiting cell growth in Hep G2 cell lines and it is therefore important to consider potential implications of this cytotoxicity in a clinical context.

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