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Cytotoxicity Test of N'-E-benzylidene benzohydrazide in UM-UC-3 and MDA-MB-231 Cell Line

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Abstract

The current anticancer therapy strategy that can be done is chemotherapy. Although cisplatin has become a common drug for the clinical treatment of solid tumors, its use has been largely limited due to the inherent and acquired resistance and severe toxic side effects in normal tissues. The search for anticancer drugs continues to this day. Hydrazone compounds are a group of organic compounds derived from reactions between aldehydes or ketones and hydrazine. Hydrazone contains an azomethine bond -CH=N-NH- which has anticancer and antitumor activity. This study aims to determine the cytotoxicity of N'-E-benzylidene benzohydrazide compounds on UM-UC-3 and MDA-MB-231 cancer cell lines. Cytotoxicity analysis was carried out using CCK-8 to obtain the Inhibitory Concentration (IC_{50}) value with a nonlinear regression test using GraphPad Prism 8.3.0 for Windows software. The compound N'-E-benzylidene benzohydrazide was shown to have a lower IC_{50} value in the MDA-MB-231 cell line at a concentration of 100 µM namely 482 µM. Therefore, the study concludes that N'-E-benzylidene benzohydrazide is still toxic to cancer cell lines, particularly UM-UC-3 and MDA-MB-231 cell lines. More research will be needed before this compound may be used as an anticancer medication in the future.

Keywords: Cancer; Cytotoxic; CCK-8; Hydrazone

1. Introduction

The current anti-cancer therapy strategy that can be employed is chemotherapy [1]. However, there are still challenges in cancer treatment due to therapeutic resistance [2,3]. This resistance occurs when cancer develops resistance to treatments such as chemotherapy through various mechanisms [4]. Cancer or malignant tumor is an abnormal condition of a group of cells that do not follow the functional roles of normal cell distribution and develop through uncontrolled pathways. Cancer cells do not respond to cell cycle stimuli and can reproduce themselves indefinitely, causing death. The cause of death from cancer is generally caused by the consequences of the spread of cancer cells to other body tissues, which is referred to by the term metastasis [5].

In research on cancer treatment, there is cisplatin, a chemotherapeutic agent that has been used to treat various human malignancies and many solid tumors. However, cisplatin's use has been largely limited due to inherent and acquired resistance and severe toxic side effects on normal tissues. These toxic side effects significantly diminish patients' quality of life [6]. Therefore, hydrazone compounds have been discovered as potential substitutes for cisplatin. Hydrazone is an organic compound that can be utilized as an anticancer agent because it can act as an androgen receptor inhibitor and tyrosine kinase inhibitor [7]. Various hydrazone derivatives have been synthesized with pharmacological activities, particularly anticancer properties [8]. Benzohydrazide is a compound with C=O, C-N, and N=N groups [9], that exhibit

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various biological activities such as antioxidant, antitumor, and anticancer effects [10,11]. Furthermore, benzaldehyde has shown some antitumor activities [12].

Several studies have been conducted on cytotoxicity, including the research conducted by Aydin et al examined the hydrazone compound has an IC_{50} value between 24.02 to 137.50 µM which was tested in MDA-MB-231 cell line [13]. Furthermore, Kumar et al. demonstrated that 14 hydrazone derivatives have cytotoxic activity against MDA-MB-231 cell lines which are 1.0 to >100 µM [14]. Another study conducted by Mladenova et al. showed that MDA-MB-231 cell lines were tested with 11 hydrazone complex ligands. The results of the test showed that the ligand had an IC50 value from 2.80 ± 2.7 to >200 µM [15]. Ahmed et al. also proved that 14 hydrazone derivatives have cytotoxic activity against MDA-MB-231 cell lines which are 7.9 ± 0.13 to >100 µM [16]. Therefore, further studies are needed to investigate the cytotoxicity of N'-E-benzylidene benzohydrazide in UM-UC-3 and MDA-MB-231 cell lines.

2. Material and Methods

This study using the DMEM culture medium for MDA-MB-231 cell line was prepared from 10% FBS and 1% Penicillin-streptomycin dissolved in a glass bottle. Furthermore, Dulbecco's Modified Eagle's Medium (DMEM) was added. MEM culture medium for UM-UC-3 cell line was prepared from 10% FBS; 1% Penicillin-streptomycin; and 1% Sodium pyruvate; which were dissolved in a glass bottle. Next, Minimum Essential Medium (MEM) was added.

Stock solutions of N'-E-benzylidene benzohydrazide at a concentration of 100.000 µM was made by dissolving 0.0250 grams of N'-E-benzylidene benzohydrazide powder with 1000 µl of DMSO solvent in a microtube. Then the stock solution was aliquoted into 5 microtubes containing 10 µL each and stored in a 4 ℃ refrigerator. The remaining stock solution was stored in a -80 ℃ refrigerator.

For the cytotoxic assay, a 96-Well plate with a density of $5x10^3$ cells in 100 µL medium. Cells were treated with the highest compound concentrations of 100 µM and 50 µM and incubated for 48 hours. After incubation, each well was treated with 10 µL of CCK-8. Then, cells were read on the Elisa Reader at 450 nm.

The data obtained from the study will be processed, edited, tabulated, and cleaned. Processing of data analysis in the cytotoxicity test was carried out using GraphPad Prism 8.3.0 software for Windows for Inhibitor Concentration 50 (IC_{50}) value with a nonlinear regression test.

3. Result

Based on the study that has been done, the results of Cytotoxicity testing was carried out by giving the compound N'-E-benzylidene benzohydrazide to the MDA-MB-231 cell line with the highest concentration of 100 µM and 50 µM. At a concentration of 100 µM has a concentration series of 100 µM; 20 µM; 4 µM; 0.8 µM; 0.16 µM; 0.032 µM; and 0.0064 µM. While at a concentration of 50 µM has a concentration series of 50 µM; 10 µM; 2 µM; 0.4 µM; 0.08 µM; 0.016 µM; and 0.0032 µM. Then using DMSO levels of 0.1% and cell control. The cytotoxicity test of N'-E-benzylidene benzohydrazide against MDA-MB-231 cells was carried out using CCK-8 and the inhibition graph can be seen in Figures 1 and 2.

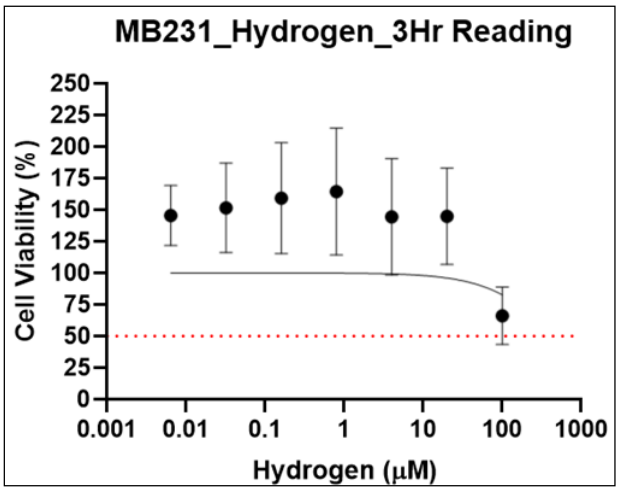

Figure 1 Percentage induction of cytotoxic activity at MDA-MB-231 cell line used 100 µM as a higher concentration

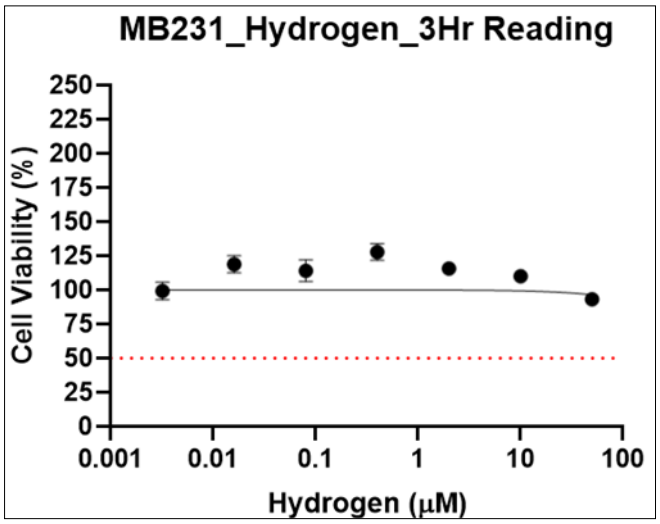

Figure 2 Percentage induction of cytotoxic activity at MDA-MB-231 cell line used 50 μM as a higher concentration

After obtaining the absorbance value at each serial concentration in the ELISA Reader, the data was tested using GraphPad Prism 8.3.0. From the inhibition graph, it is known that the IC_{50} for the compound N'-E-benzylidene benzohydrazide is 482 μM at a concentration of 100 μM, and the IC_{50} for the compound N'-E-benzylidene benzohydrazide is 1334 μM at a concentration of 50 μM.

Cytotoxicity testing was carried out by giving the compound N'-E-benzylidene benzohydrazide to UM-UC-3 cell line with the highest concentration of 100 μM and 50 μM. At a concentration of 100 μM has a concentration series of 100 μM; 20 μM; 4 μM; 0.8 μM; 0.16 μM; 0.032 μM; and 0.0064 μM. While at a concentration of 50 μM has a concentration series of 50 μM; 10 μM; 2 μM; 0.4 μM; 0.08 μM; 0.016 μM; and 0.0032 μM. Then using DMSO levels of 0.1% and cell control. The cytotoxicity test of N'-E-benzylidene benzohydrazide against UM-UC-3 cell line was conducted using CCK-8 and the inhibition graph can be seen in Figure 3 and 4.

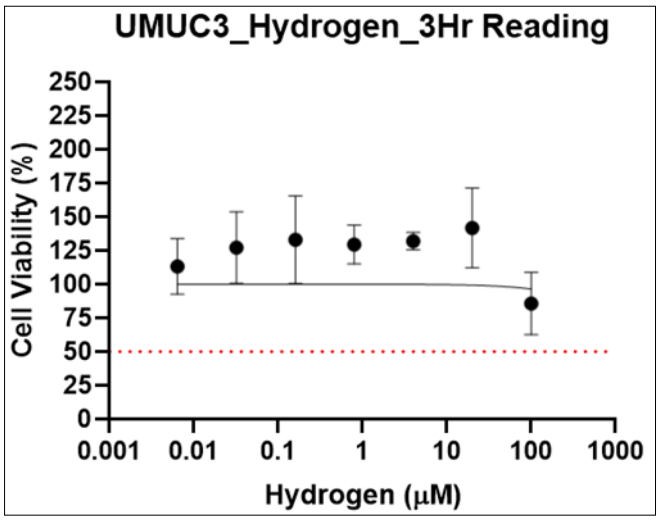

Figure 3 Percentage induction of cytotoxic activity at UM-UC-3 cell line used 100 μM as a higher concentration

After obtaining the absorbance value at each serial concentration in the ELISA Reader, the data was tested using GraphPad Prism 8.3.0. from the inhibition graph, the IC_{50} for the compound N'-E-benzylidene benzohydrazide was 2719 μM at the highest compound concentration of 100 μM and the IC_{50} for the compound N'-E-benzylidene benzohydrazide was 1027 μM at the highest compound concentration of 50 μM.

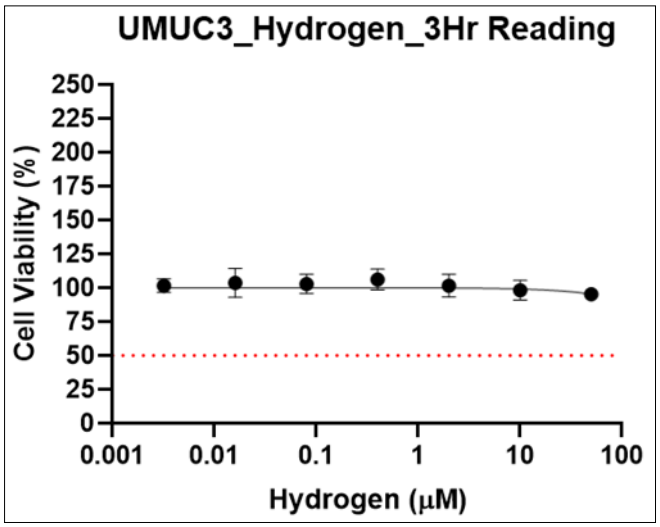

Figure 4 Percentage induction of cytotoxic activity at UM-UC-3 cell line used 50 μM as a higher concentration

4. Discussion

Based on the method steps, Cytotoxic tests that have been carried out against MDA-MB-231 and UM-UC-3 cell lines obtained the percentage of living cells that can be seen in Figures 1-4. The compound N'-E-benzylidene benzohydrazide still does not qualify as a possible candidate for an anticancer agent. This is a result of the N'-E-benzylidene benzohydrazide compound's persistently high IC_{50} value of 2719 μM, 1027 μM, 482 μM, and 1334 μM. When the IC_{50} value is low, it indicates that the drug is effective even at lower concentrations, causing less systemic toxicity when administered to the patient [17].

Cytotoxicity testing of the compound against MDA-MB-231 cell line and UM-UC-3 cell line was performed with CCK-8 Assay. By using highly water-soluble tetrazolium salts, the CCK-8 assay shows more sensitivity in detecting cellular activity compared to other tetrazolium salt-based assays such as MTT. In the CCK-8 assay, WST-8 dye (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) is reduced by dehydrogenase in the cell, resulting in a water-soluble, orange-colored product known as formazan. The quantity of formazan dye produced by cellular dehydrogenase is directly related to the number of living cells [18]. The results obtained in the form of absorbance values derived from the ELISA Reader were then analyzed using GraphPad to obtain the inhibitor graph and IC_{50} value. IC_{50} stands for half-maximal inhibitory concentration. It is a widely used and informative measure to measure the effectiveness of drug. This important value indicates the amount of drug needed to inhibit a biological process by 50%, providing valuable information about the potency of inhibitors in pharmacological research [19]. Furthermore, drugs can be compared by analyzing their IC_{50} values, which represent the drug concentration required to inhibit half of the growth of a tumor cell colony. This comparison makes it possible to determine which drug is more effective [17].

5. Conclusion

Based on study that has been carried out, the compound N'-E-benzylidene benzohydrazide is still toxic to cancer cell lines, particularly UM-UC-3 and MDA-MB-231 cell lines. More research will be needed before this molecule may be used as an anticancer medication in the future.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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