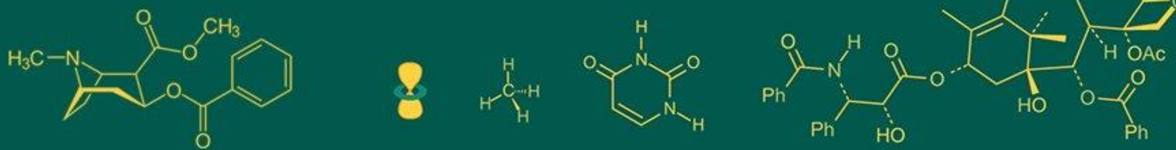

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(1): 846-852
www.biochemjournal.com
Received: 14-10-2024
Accepted: 17-11-2024

Noor M Naser
Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

Dakhil Zughayir Mutlaq
Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

Ali AA Al-Shawi
Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

Corresponding Author:
Ali AA Al-Shawi
Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

Apoptosis and ROS assessment of a 2-thioxoimidazolidin-4-one analogue using HepG2 cells

Noor M Naser, Dakhil Zughayir Mutlaq and Ali AA Al-Shawi

DOI: https://doi.org/10.33545/26174693.2025.v9.i1k.3648

Abstract
This study involved evaluation of 12 2-thioxoimidazolidin-4-one analogue 6a-6g and 7a-7e against HepG2 liver cells using the MTT assay, apoptosis, and reactive oxygen species ROS experiments. The compounds 6a-6g and 7a-7e showed various IC_{50} values (29.44, 27.68, 600.54, 597.85, 135.83, 105.28, 115.70, 68.90, 300, 36.64, 18.29, and 15.49) µM, respectively, and the compound 7e has the lower IC_{50} value 15.49 µM. The IC_{50} value of compound 7e showed significant effect with apoptosis and ROS. Therefore, it's shown promising results with merit investigations.

Keywords: Thiohydantoin derivatives, apoptosis, ROS, liver cancer

Introduction
Liver cancer is considered the most common and dangerous type of cancer in the world due to its rapid growth that begins as a normal infection that can develop into cirrhosis and then cancer due to the absence of clear symptoms until it reaches advanced stages, which leads to an increase in the number of deaths [1]. The liver is constantly exposed to many external toxins from food in the intestines, which are widely used as cancer-promoting chemicals, such as preservatives for many foods that act as bacterial inhibitors. Due to heat and stomach acidity, they are transformed into highly toxic substances that cause liver infections and immune and physical damage that causes cancer [2]. It ranks third among the types of cancer in the world, as the World Health Organization stated that the number of deaths resulting from liver cancer reached 850,000 in 2020, representing 90% of primary liver cancer cases [3]. Although studies have shown that the liver has the ability to regenerate damaged cells and heal wounds in a clear way, it is linked to a special control system and a complex group of factors, including growth factors [4]. Regulating the path of molecular signals that are linked to the cancer cell greatly helps in discovering new treatments and targets, and discovering treatments that target the proteins that make up cancer cells works to reduce toxicity to the rest of the different cells [5]. Thiohydantoin derivatives have been characterized by great interest in the discovery of therapeutic drugs due to their diverse biological properties. Their effectiveness has been proven against different types of diseases, specifically with various tumors due to the chemical structure of these compounds, which made them a key to predicting the mechanism of many chemical reactions [6]. They have shown a great ability to overcome most microbes and as antioxidants and anti-tumors [7]. The thiohydantoin ring is considered effective compounds for inhibiting EGFR receptors, as they have been approved as clinical drugs due to their high ability to control cytotoxicity [8]. Among the drugs included in their composition are the thiohydantoin cyclase, enzalutamide and nilutamide, as antagonists of androgen receptors [9]. The aim of this research was to evaluate the bioactive compounds against HepG2 cells using MTT assay, apoptosis, and ROS.

Materials and Methods
The series of 2-thiohydantoin derivatives 6a-6g and 7a-7e were prepared by Naser *et al.* [10], and used for anti-liver evaluation HepG2 cells.

Evaluation of anticancer activity
HepG2 is a human liver cancer cell line that was given by the National Cell Bank of Iran

(Pasteur Institute, Iran). Some medicines (100 U/ml penicillin and 100 μg/ml streptomycin) and RPMI-1640 (Gibco) were added to DMEM medium (Gibco) and cells were grown in them. The cells were grown at 37 °C in 5% CO_2 humidified air and were passed through trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution. Conditions and media used to grow the cells in 3D clusters were the same as those used for growing cells in a single layer.

Cell viability assay

The MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to check how much cells were growing and if they were still alive. Cells were broken down with trypsin, collected, and standardized to have a density of 1.4×10^4 cells/well. They were then seeded for 24 hours in 96-well plates that had 200 μl of fresh medium in each well. Cells that were stacked one on top of the other were treated with 100–6.25 μg/ml of substances for 24 hours at 37 °C in 5% CO. After 24 hours, the supernatant was taken out and 200 μl/well of MTT solution (0.5 mg/ml in PBS) was added to the plate. It was then left to sit at 37 °C for another 4 hours. Dimethyl sulfoxide (100 μl per well) was added to the cell supernatant to make an MTT solution. At 37 °C, the cells were shaken until the crystals were gone. An ELISA reader was used to measure absorbance at 570 nm to find out if the cells were still alive. "Typical Wave xs2 Biotech, United States of America" Dose-response plots were used to find the IC50 values [11].

Apoptosis assay

The Annexin V-FITC Apoptosis Kit was used to find HepG2 cells that had been treated with chemical 7e and had died or gone into necrosis. The cells were treated with an IC_{50} value of 7e 15.49 μg/ml for 4 hours. They were then collected, washed well, and labelled with PI and FITC according to the instructions that came with the kit. To sum up, 5×10^5 cells were collected and centrifuged after being treated with IC_{50} value for 24 hours. Control cells that had not been treated were also used for 10 minutes. After that, the pellet was mixed again with 500 mM 1X binding buffer, 5 μl of annexin V-FITC, and 5 μl of propidium iodide (50 μg/ml). It was then left to sit at room temperature for 5 minutes. The marked cells were looked at with a FACS analyser (flow cytometer, Partec PAS, Germany) after they had been incubated. The spread of differently labelled cells was found by getting signals from cells that were stained with PI and Annexin V-FITC using the FITC signal reagent (FL1) and from cells that were stained with Annexin V-FITC using the FL3 signal reagent [11].

Reactive oxygen species assay

2′,7′-dichlorodihydrofluorescein diacetate (DCFH2-DA) was used in flow cytometry to measure ROS generation. It's easy for this color to get into cells, where it is broken down by intracellular esterase into $DCFH_2$, which still stays inside the cells. Hydrogen peroxide or low molecular weight peroxides made by cells can change DCFH2 into 2', 7′-Dichlorofluorescein (DCF), which is a very bright chemical. So, the strength of the fluorescence was related to the amount of hydrogen peroxide the cells made. The IC_{50} concentration of the substance was used to treat HepG2 cells (1×10^5 cells/well) for 12 hours. About 30 minutes before the experiment was over, cells were broken up with trypsin and centrifuged for 5 minutes. The pellet was then cleaned with 1 ml of PBS and treated with DCFH2-DA. It was then put away in the dark. Compare two excitation/emission fluorescence bands (485–495 nm and 525–530 nm) after 30 minutes to see how bright the fluorescence was [11].

Results and Discussion

Anticancer activity

The discovery of anti-liver cancer drugs is considered one of the most important achievements facing medicine recently due to the large spread of this type of cancer. During the study, the MTT of 6a-6g and 7a-7e against HepG2 cancer cells was measured and the IC_{50} values were calculated as shown in Table 1, Figure 1, and Figure 2.

The compound 7e showed the lowest value compared to the rest of the compounds because the compound contained a chlorine atom and NO_2 group, this reason for the difference in the effectiveness of these compounds is due to the difference in functional groups. The results of IC_{50} effect of compound 7e on the apoptosis and ROS were determined using flow cytometry. As shown in Figure 3A (untreated cells) live cells Q4 percentage is 97%, while in Figure 3B (treated cells) live cells Q4 is 39.3%. Necrosis cells Q3 percentage is 2.48% in untreated cells, while necrosis cells Q3 percentage 60.6% in treated cells. Apoptosis results are enhanced by the ROS results, Figure 3C, untreated cells showed 97.7%, while treated cells in Figure 3D showed decreasing to 69.3%.

These results indicate the function of compound 7e against HepG2 liver cancer cells due to the structure of functional groups, which request for a merit development and investigations to approve the potential activity of compound 7e.

Table 1: Shows the IC_{50} values for the compounds 6a-6g and 7a-7e against HepG2 cancer cells

Symbol	Chemical structure	HepG2 cells IC_{50} in µM
6a		29.44
6b		27.68
6c		600
6d		597
6e		135
6f		105
6g		115.70
7a		86.90
7b		300
7c		36.64
7d		18.29
7e		15.49

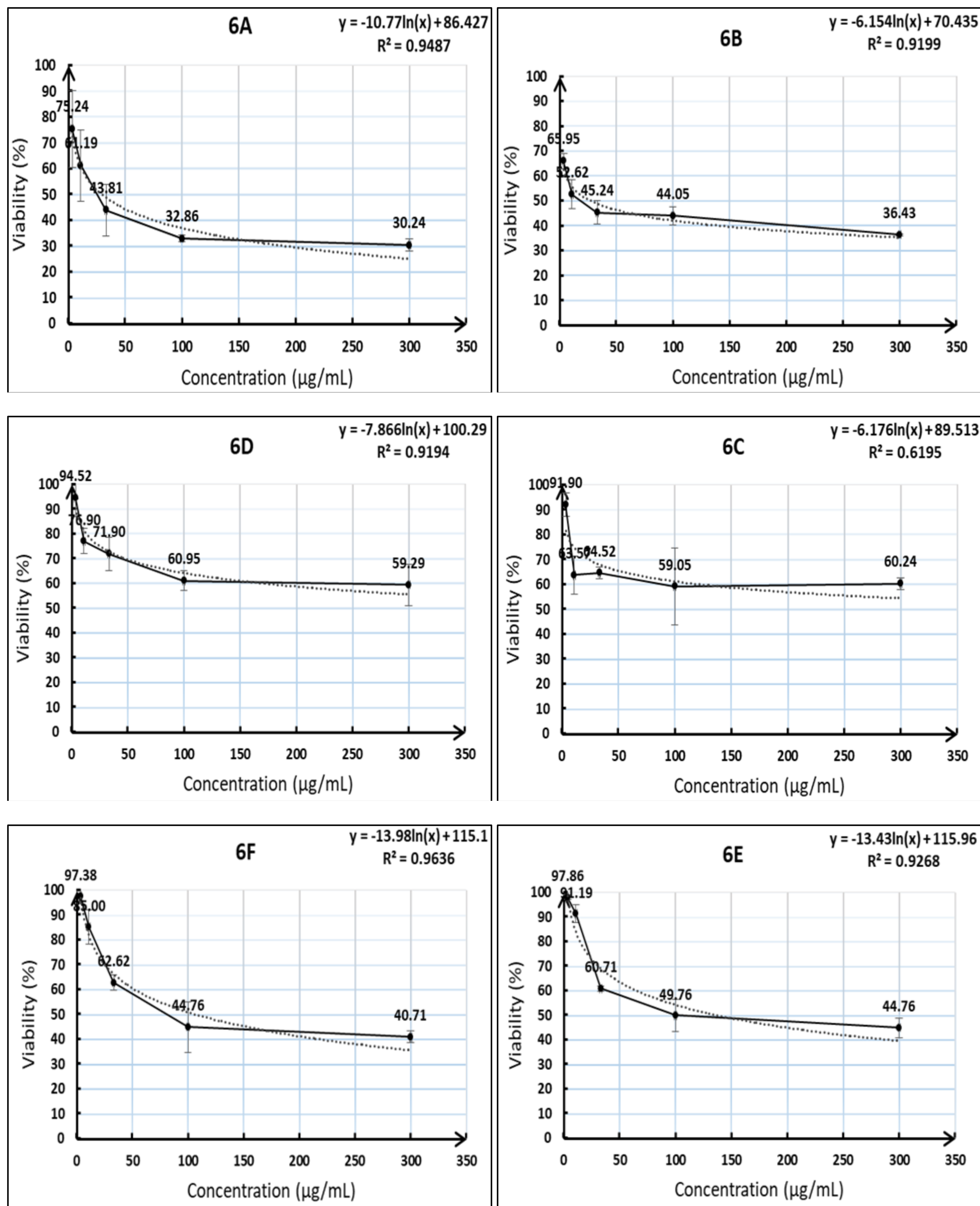

Fig 1: Showed the IC_{50} calculation graph of the prepared compounds 6a-6e against HepG2 cancer cells.

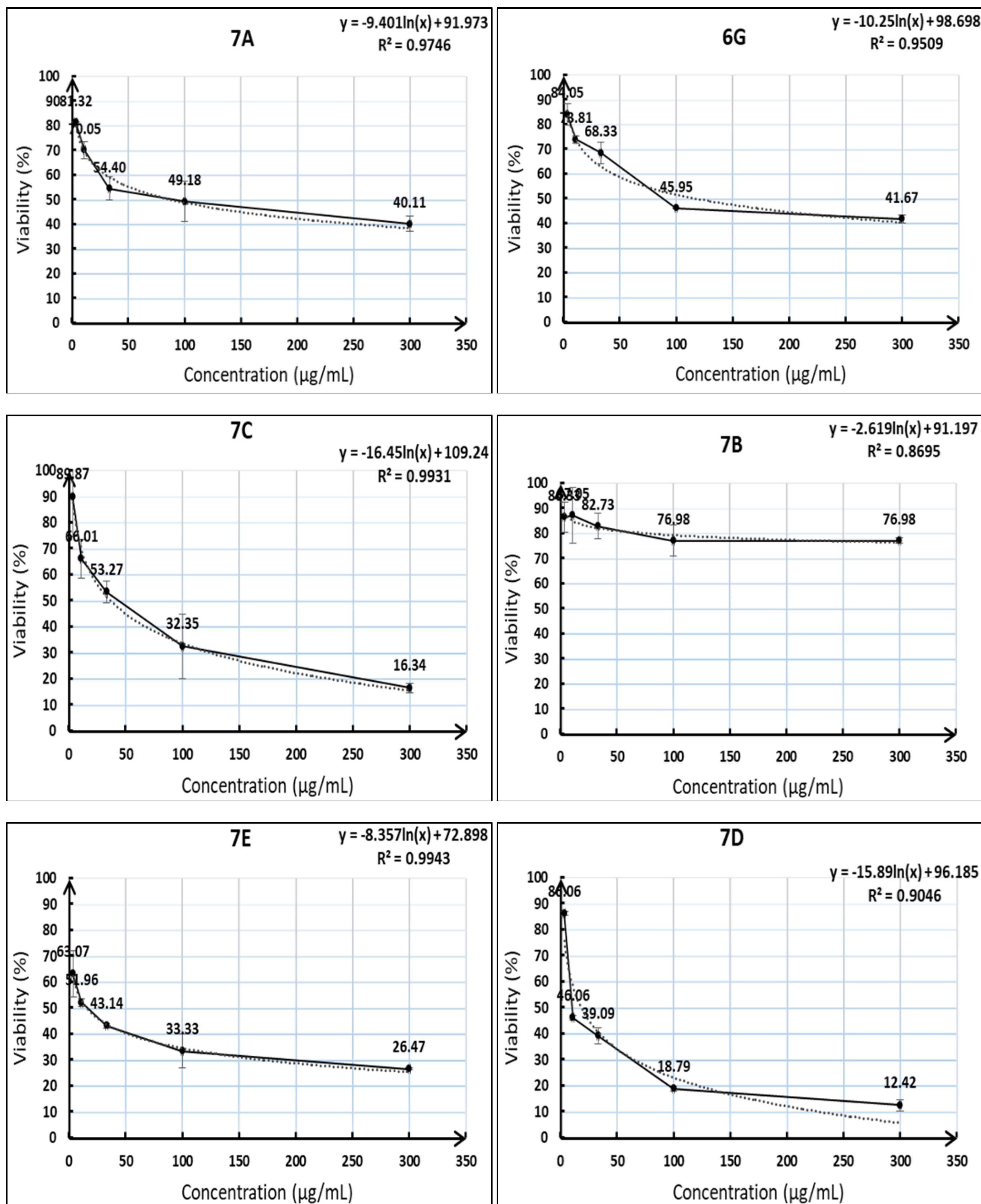

Fig 2: Showed the IC_{50} calculation graphs of the prepared compounds (6f) and 7a-7e against HepG2 cancer cells.

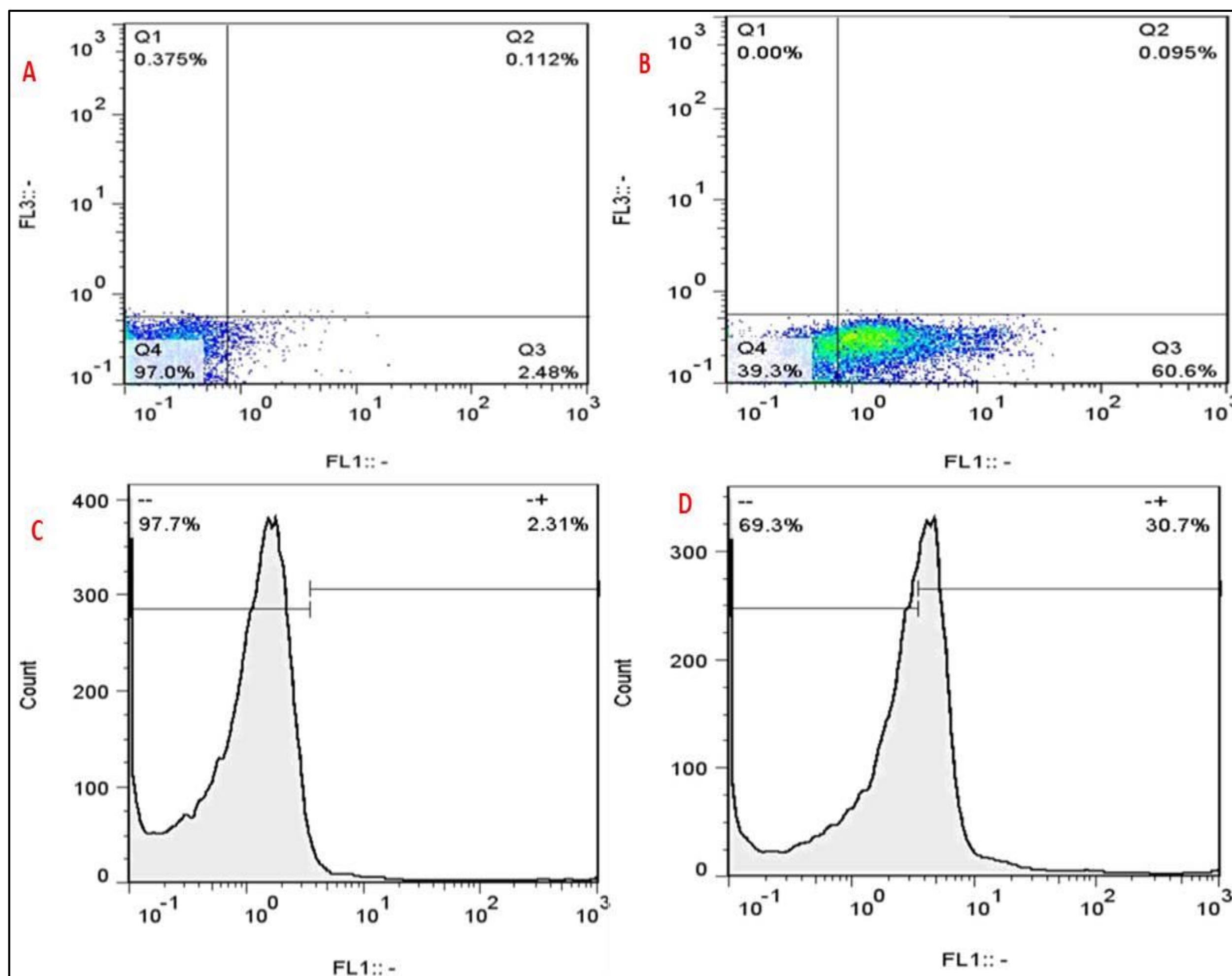

Fig 3: Analysis using the flow-cytometry method revealed: A. Cells that had not been treated and had an IC_{50} of 7e were studied using apoptosis. B. Treated cells with IC_{50} value of compound 7e analyzed using apoptosis. C. Untreated cells with IC_{50} of compound 7e analyzed using ROS. D. Treated cells with IC_{50} value of compound 7e analyzed using ROS.

In addition, the other compounds also can be investigated especially, compounds 6a, 6b, 7c, and 7d. The Count-FL1 map is used to figure out how much ROS is being made. In the treated cells with 7e compound when compared to control cells the FL1 peak has shifted to the right. The more the peak shifts to right indicates that more of the cell population has produce fluorescent light which is proportional to increase in ROS production. The anticancer activity of compound 7e due to the presence of the NO_2 group and chlorine group, which has the ability to hydrogen bond with free radicals or donate a proton or electron from a heteroatom to free radicals and thus end their role, so they become stable and cannot attack other molecules and thus stop the oxidation reaction that leads to increased inhibition of human liver cancer cells (HepG2) [12-16].

Conclusions

IC_{50}, apoptosis, and cell cycle studies have revealed the significance of compounds 6a-6g and 7a-7e in HepG2 cancer cells. This merits further exploration in order to pinpoint its possible function. Thus, developing additional compound 7e derivatives or combining it with other compounds may improve its function and biological applicability for a variety of ailments.

Acknowledgement

This research project is funded by the Ministry of Higher Education and Scientific Researches, University of Basrah, College of Education for Pure Sciences, Department of Chemistry, Iraq, and it is part of the Ph.D program's graduation requirements.

References

1. Akree LS, Amin ZA, Ahmad HO. In silico and in vivo hepatoprotective activity of the synthesized 5-benzylidene-2-thiohydantoin against diethylnitrosamine-induced liver injury in a rat model. Sci Rep. 2023;13(1):4681.
2. Lin ZY, Chuang WL. Contrary influence of clinically applied sorafenib concentrations among hepatocellular carcinoma patients. Biomed Pharmacother. 2017;86:27-31.
3. AbdulJabar LA, Mutalq DZ, Al-Shawi AA. Synthesis of novel 2-thioxo-4-imidazolidinone derivatives and evaluation of their antibacterial and antifungal activities. Egypt J Chem. 2021;64(6):3059-3067.
4. Carretero J, Obrador E, Esteve JM, Ortega A, Pellicer JA, Sempere FV, *et al.* Tumoricidal activity of endothelial cells: inhibition of endothelial nitric oxide production abrogates tumor cytotoxicity induced by hepatic sinusoidal endothelium in response to B16 melanoma adhesion in vitro. J Biol Chem. 2001;276(28):25775-25782.
5. Kim E, Viatour P. Hepatocellular carcinoma: old friends and new tricks. Exp Mol Med. 2020;52(12):1898-1907.

6. Elhady HA, El-Sayed R, Al-Nathali HS. Design, synthesis and evaluation of anticancer activity of novel 2-thioxoimidazolidin-4-one derivatives bearing pyrazole, triazole and benzoxazole moieties. Chem Cent J. 2018;12:1-13.
7. Albakhit SD, Mutlaq DZ, Al-Shawi AA. Antibacterial, antifungal, and antitumor properties of 2,5-pyrrolidinedione derivatives. Chem Afr. 2023;6(6):2933-2944.
8. AbdulJabar LA, Al-Shawi AA, Mutlaq DZ. Anti-liver and anti-breast cancer activities of 2-thioxo-4-imidazolidinone derivatives. Med Chem Res. 2021;30(10):1943-1953.
9. Al-Shawi AA, El-Arabey AA, Mutlaq DZ, Eltayb WA, Iriti M, Abdalla M. Study on molecular anti-tumor mechanism of 2-thiohydantoin derivative based on molecular docking and bioinformatic analyses. Curr Top Med Chem. 2023;23(6):440-452.
10. Naser NM, Mutlaq DZ, Al-Shawi AAA, *et al*. Synthesis, characterization, antibacterial, and molecular docking and dynamics studies of novel 2-thioxoimidazolidin-4-one analogue. Chem Afr. 2024. https://doi.org/10.1007/s42250-024-01040-y
11. Thawini HK, Al-Shawi AA. A polysaccharide of *Ziziphus spina-Christi* L., and its silver nanoparticles induced reactive oxygen species and late apoptosis of liver cancer cells. Nanomed Res J. 2021;6(3):237-247.
12. Mahdy AR, Alfaifi MY, El-Gareb MS, Farouk N, Elshaarawy RF. Design, synthesis, and physicochemical characterization of new aminothiohydantoin Schiff base complexes for cancer chemotherapy. Inorg Chim Acta. 2021;526:120504.
13. Jadhav PM. A review on biological activities of Schiff base ligand and their metal complexes. J Chem Tech. 2020;13:217-221.
14. Ismail LA, Zakaria R, Hassan EM, Alfaifi MY, Shati AA, Elbehairi SEI, *et al*. Novel imidazolium-thiohydantoin hybrids and their Mn (III) complexes for antimicrobial and anti-liver cancer applications. RSC Adv. 2022;12(44):28364-28375.
15. Cho S, Kim SH, Shin D. Recent applications of hydantoin and thiohydantoin in medicinal chemistry. Eur J Med Chem. 2019;164:517-545.
16. Hassan AY, Said MM, Sarg MT, Al-Zahabi HS, Hussein EM. Utility of 2-thiohydantoin derivatives in the synthesis of some condensed heterocyclic compounds with expected biological activity. Life Sci J. 2013;10(1):16-19.