Original Article

DOI: 10.4274/haseki.galenos.2025.9962
Med Bull Haseki 2024;62:265-271

Modulation of Apoptosis and ER Stress Markers in Hepatocellular Carcinoma Cells by Irinotecan, Hesperidin, and Piperine

Serkan Sen*, Kardelen Kocaman Kalkan**, Canan Yilmaz**, Sefa Celik***

*Afyonkarahisar Health Sciences University, Ataturk Vocational School of Health Services, Deparment of Medical Laboratory Techniques, Afyonkarahisar, Turkey

**Gazi University Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey

***Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Biochemistry, Afyonkarahisar, Turkey

Abstract

Aim: Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, and there is a pressing need to explore novel therapeutic strategies that enhance the efficacy of existing treatments. This study aims to investigate the effects of irinotecan (IRT), hesperidin (HSP), and piperine (PIP) on HCC (HepG2), focusing on their modulation of apoptosis-related genes and endoplasmic reticulum (ER) stress markers.

Methods: This study is an in vitro experimental study. IC50 values for IRT, HSP, and PIP were determined using MTT cell viability assays. Researchers performed total RNA extraction and quantitative PCR to assess mRNA levels of *Bad, Bax, and p53* (apoptosis-related genes) and ATF4, CHOP, and GRP78 (ER stress markers).

Results: Irinotecan significantly upregulated the expression of *Bad, Bax, and p53* genes, as well as ER stress markers such as ATF4, CHOP, and GRP78. Hesperidin-enhanced apoptotic gene expression and exacerbated ER stress. Piperine attenuated IRT-induced apoptosis and suppressed ER stress markers.

Conclusion: Combining IRT with HSP enhanced apoptosis and ER stress in HepG2 cells, suggesting synergistic potential against HCC. Conversely, IRT combined with PIP reduced apoptotic response and ER stress markers, possibly compromising IRT's efficacy. These findings highlight complex interactions between chemotherapeutic agents and natural compounds, warranting further exploration in combination therapies.

Keywords: Irinotecan, hesperidin, piperine, carcinoma, hepatocellular, apoptosis, endoplasmic reticulum

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer type globally and the third leading cause of cancer-related deaths, with an average of more than 740,000 new diagnoses per year (1). HCC, originating from liver cells, poses a substantial global health burden primarily linked to chronic viral hepatitis infections, alcohol-related liver diseases, and non-alcoholic fatty liver disease (NAFLD/NASH). Molecular studies highlight chromosomal instability, genetic mutations, and epigenetic changes as pivotal in HCC pathogenesis, influencing oncogene activation and tumor suppressor gene inactivation. Cellular processes such as enhanced proliferation, impaired apoptosis, increased angiogenesis, and metastatic potential further underscore the complexity of HCC progression (2). Immune responses within the tumor microenvironment play a critical role in HCC development and treatment outcomes, paving the way for innovative

Address for Correspondence: Serkan Sen, Afyonkarahisar Health Sciences University, Ataturk Vocational School of Health Services, Deparment of Medical Laboratory Techniques, Afyonkarahisar, Turkey
E-mail: serkansen07@gmail.com **ORCID:** orcid.org/0000-0002-2884-4753
Received: 01.06.2024 **Accepted:** 17.01.2025

therapies like immunotherapy (3). Understanding these multifaceted aspects is crucial for advancing effective management strategies against HCC.

Irinotecan (IRT) is one of the selective topoisomerase 1 (Topo1) inhibitors, including camptothecin, topotecan, idarubicin, daunorubicin, doxorubicin, and etoposide. Topo1 is an enzyme that alleviates torsional strain in DNA by inducing temporary single-strand breaks. Irinotecan, a Topo1 inhibitor, prevents the religation of these breaks, leading to DNA damage and ultimately inducing apoptosis in cancer cells. This mechanism underscores the therapeutic efficacy of IRT in cancer treatment, particularly in targeting rapidly proliferating cells. Although IRT was one of the most important drugs in the treatment of colon cancer for approximately 15 years between 1994 and 2008, its medical use continues today (4). Irinotecan causes human HCC cells to undergo apoptosis by activating p53 with its active metabolite. Irinotecan induces apoptosis in cancer cells by altering gene expression. Key genes involved in this process include p53, BAX/BCL-2, caspases, and NF-κB. The effects of IRT on gene expression promote cell death and inhibit tumor growth.

Flavonoids can be categorized into six primary classes based on their structure: flavan-3-ols, flavones, flavonols, flavanones, isoflavones, and anthocyanins (5). Among these subclasses, hesperidin (HSP) is identified as a flavanone compound. Hesperidin and its derivatives are characteristic compounds found in citrus fruits belonging to the Rutaceae family, including orange (Citrus sinensis), grapefruit (Citrus paradisi), tangerine (Citrus reticulata), lime (Citrus aurantifolia), and lemon (Citrus limon) (6). There have been many recent studies showing that HSP has anticancer activity (7). There are many studies showing that piperine (PIP), an alkaloid isolated from the piper nigrum plant, has an anticancer and enhancing effect of existing chemotherapeutics (8).

Hesperidin, a flavonoid, has been associated with various health advantages and has shown potential effects in regulating the expression of pro-apoptotic genes such as Bad, Bax, and p53. Piperine, an alkaloid found in spices, is recognized for its anti-cancer effects, with research focusing on its ability to modulate the expression of apoptosis-related genes like Bad, Bax, and p53 (9). These studies provide foundational insights into understanding the cellular effects of HSP and PIP and evaluating their potential therapeutic applications.

In recent years, the combination of chemotherapeutic agents with natural compounds has gained attention as a promising approach to enhance the efficacy of cancer treatments. However, the combination of IRT with natural compounds like HSP and PIP has not been extensively investigated. This study aims to address this research gap by evaluating the effects of IRT in combination with HSP and PIP in hepatocellular carcinoma cells, providing new insights into potential therapeutic strategies for improving cancer treatment outcomes.

Methods

Compliance with Ethical Standards

This study was conducted using commercially obtained HepG2 cell lines. No patient samples or primary cell cultures were used. Therefore, ethical approval was not required.

Chemicals and Reagents

Irinotecan and HSP were purchased from Cayman Chemical Company (Michigan, USA, Cat No: 14180); PIP was purchased from Sigma-Aldrich (Darmstadt, Germany, Cat No.: P49007) (Michigan, USA, Cat No: 18646). HCC cell line (HepG2) was purchased from American Type Culture Collection (HB-8065, Manassas, VA, USA). All primers were synthesized by Bioligo Biotechnology (Ankara, Turkey).

Cell Culture

The HepG2 cell line was cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum, 5% L-Glutamine, and 1% penicillin-streptomycin. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

Determination of IC50 doses with the MTT Cell Viability Test

Cells were seeded at a density of 10^4 cells per well in 96-well plates. Cells were treated with different concentrations of IRT (1 µM, 10 µM, 25 µM, 50 µM, 100 µM, 250 µM, 500 µM, and 1000 µM) and different concentrations of HSP and PIP (1 µM, 10 µM, 50 µM, 100 µM, 200 µM, 400 µM, 800 µM, and 1000 µM). Then, 10 µL of MTT solution (5 mg/mL, SERVA, Heidelberg, Germany) was added to each well and incubated for 4 hours at 37 °C with 5% CO_2. After the incubation period, 100 µL of dimethyl sulfoxide (DMSO) was added to each well, and the absorbance of each well was measured at 570 nm using an automatic multiplate reader (Epoch, Biotek, USA). IC50 values were calculated using GraphPad Prism version 8.0.1 (GraphPad Software, Inc., CA, USA) (10). Each analysis was performed in triplicate.

Total RNA Extraction, Reverse Transcription, and Quantitative Polymerase Chain Reaction

After completion of the 48-hour incubation, cells were harvested and washed with cold PBS. For mRNA expression level, total RNA was isolated with the GeneJET RNA Purification Kit (Thermo Scientific Catalog No.: K0731). The quantity and purity of isolated RNAs were analyzed using the Epoch Take3 plate instrument (Agilent, USA). Then, complementary DNA (cDNA) synthesis was

performed according to the manufacturer's instructions (Biorad Cat No: BR1708891). Each 20 µL PCR reaction consisted of 1 µL of cDNA, 10 µL of 2X SYBR Green PCR Master Mix (prepared as 1X according to the manufacturer's guidelines), and specific primers, with a final concentration of 100 ng per reaction. 1 µg total RNA was used as a template in the PCR performed with reverse transcriptase. Then, 1 µL of cDNA was taken from each sample and the appropriate amount of SYBR Green PCR Master Mix was added according to the forward and reverse primer protocols. All stages were carried out under cold chain and sterile conditions. Expression levels of target genes were normalized using the housekeeping gene β-Actin. Gene expression values were then calculated with the REST2009 program by applying the ΔΔCt method using the equation RQ=2−ΔΔCt, according to (11). Primer sequences used in PCR reactions and PCR conditions are described in Table 1. Each assay was performed in quadruplicate.

Statistical Analysis

The data obtained within the scope of the study were analyzed using GraphPad Prism version 8.0.1 (GraphPad Software, Inc., CA, USA). The data was analyzed to determine whether it showed a normal distribution using the Kolmogorov-Smirnov test. Since the data showed normal distribution, one-way ANOVA test, one of the parametric tests, was used for the comparison of three or more groups. P<0.05 was considered the level of statistical significance.

Results

IC50 doses of bioactive compounds

It was found that IC50 values of IRT were 56.02 µM, HSP was 150.7 µM and PIP was 51.14 µM for 48 hours of treatment (Figure 1).

Findings on mRNA expression levels of genes that play a role in apoptotic processes

Compared to the control group, mRNA expression levels of the *Bad* gene increased 3.231-fold (p=0.002) in the IRT group, 8.351-fold (p=0.008) in the HSP group, and 3.044-fold (p=0.005) in the PIP group. The mRNA expression levels of *Bad* gene were also increased 5.227 times (p=0.004) in the IRT+HSP combination group and

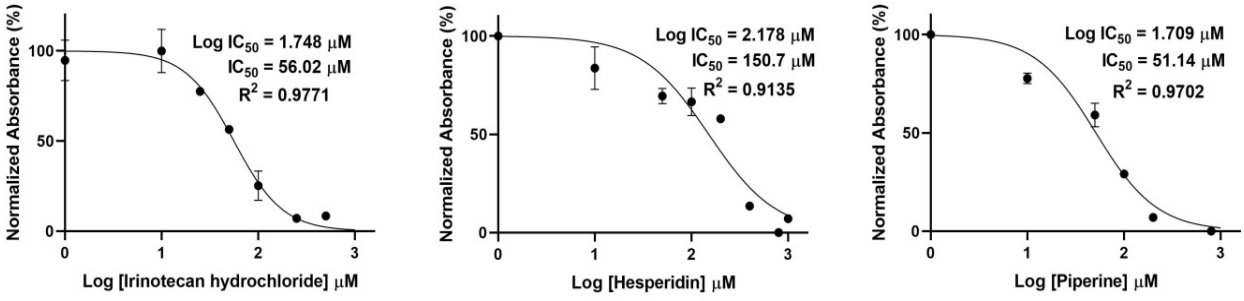

Figure 1. IC50 doses of IRT, HSP and PIP
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

Table 1. Oligonucleotide primer sequences and PCR programs

Genes	Primer sequences (5' → 3')	RT-PCR Programs	Cycle
β-Actin	F-5' CTCCATCCTGGCCTCGCTGT 3' R-5' GCTGTCACCTTCACCGTTCC 3'	95˚C-30s/60˚C-1m/72˚C-30s	35
Bax	F-5' CATGAAGACAGGGGCCCTTT 3' R-5' AAACACAGTCCAAGGCAGCT 3'	95˚C-30s/59˚C-1m/72˚C-30s	35
Caspase3	F-5' GTGCTACAATGCCCCTGGAT 3' R-5' GCTGGATGCCGTCTAGAGTC 3'	95˚C-30s/59˚C-1m/72˚C-30s	35
p53	F-5' CCTCTCCCCAGCCAAAGAAG 3' R-5' GAAGTGGGCCCCTACCTAGA 3'	95˚C-30s/59˚C-1m/72˚C-30s	35
ATF4	F- 5' GGGACAGATTGGATGTTGGAGA 3' R- 5' ACCCAACAGGGCATCCAAGT 3'	95˚C-30s/57˚C-1m/72˚C-30s	35
CHOP	F- 5' TGCTTCTCTGGCTTGGCTGAC 3' R- 5' CCAAGGGAGAACCAGGAAACGG 3'	95˚C-30s/60˚C-1m/72˚C-30s	35
GRP78	F- 5' GGTGACCTGGTACTGCTTGATG 3' R-5' CCTTGGAATCAGTTTGGTCATG 3'	95˚C-30s/57˚C-1m/72˚C-30s	35

ATF4: Activating transcription factor 4, GRP78: Glucose-regulated protein 78, CHOP: C/EBP homologous protein

1.84 times (p=0.019) in the IRT+PIP group, in comparison with the control. *Bax* gene mRNA levels were upregulated by IRT (0.492-fold, p<0.001), PIP (0.443-fold, p<0.001), and IRT+HSP (0.472-fold, p<0.003), while in the HSP group, a 0.227-fold change (p<0.006) was found to be similar to the control group (p<0.86). Compared to the control group, *p53* gene mRNA expression levels increased 1.576-fold (p=0.007) in the IRT group, 3.195-fold (p=0.007) in the HSP group, 1.428-fold (p=0.039) in the PIP group, and 2.097-fold (p=0.005) in the IRT + HSP group. The +PIP group was found to be similar to the control group (p=0.113) (Table 2, Figure 2).

Findings on mRNA expression levels of genes playing a role in ER stress

The mRNA expression levels of the *ATF4* gene showed a 1.058-fold increase (p=0.413) in the IRT group, 1.202-fold (p=0.062) in the HSP group, 1.088-fold (p=0.213) in the PIP group, and 0.963-fold (p=0.707) in the IRT+HSP group compared to controls. Conversely, *ATF4* gene expression was downregulated by 0.563-fold (p=0.006) in the IRT+PIP group compared to controls. For the *CHOP* gene, mRNA levels were significantly increased to 4.42-fold (p=0.001) in the IRT group, 4.55-fold (p=0.004) in the HSP group, 2.263-fold (p=0.014) in the PIP group, and 2.535-fold (p=0.001) in the IRT+HSP group compared to controls. No significant difference was found in the IRT + PIP group compared to controls (p=0.123). In contrast, *GRP78* gene expression increased by 1.257-fold (p=0.01) in the IRT group and decreased by 0.6-fold (p=0.009) in the IRT+PIP group, with no significant changes observed in the HSP (p=0.774) and IRT+HSP (p=0.955) groups compared to controls (Table 2, Figure 3).

Discussion

The existence of IRT resistance is known, especially in the treatment of colon cancer (12,13) and HCC (14), negatively affecting treatment strategies. Therefore, there is a need for research that will enhance the treatment efficacy of IRT and make the cells more chemosensitive during treatment.

Irinotecan application alone increased CHOP and GRP78 mRNA expression levels, which are ER stress markers (p=0.001 and p=0.01, respectively), compared to the control group, but had no significant effect on ATF4 (p=0.062). In addition, while IRT application alone

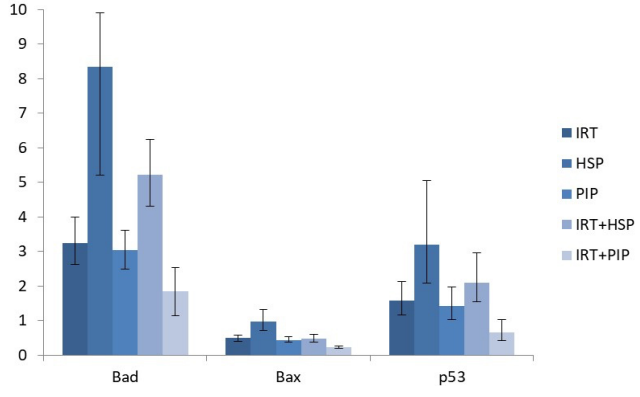

Figure 2. mRNA expression levels of apoptosis-related genes
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

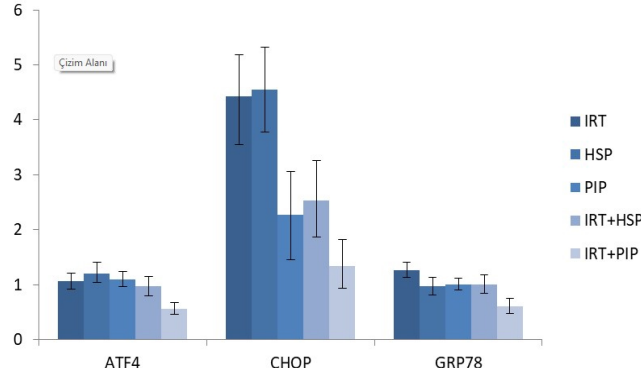

Figure 3. mRNA expression levels of ER stress-related genes
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

Table 2. Comparision of mRNA expression levels of genes

Gene expression levels						
Groups	**Apoptosis related genes**			**ER stress related genes**		
	Bad	**Bax**	**P53**	**ATF4**	**CHOP**	**GRP78**
IRT	3,231**	0.492***	1,576**	1,058	4,42**	1,257*
HSP	8,351**	0.978	3,195**	1,202	4,55**	0.975
PIP	3,044**	0.443***	1,428*	1,088	2,263*	0.999
IRT+HSP	5,227**	0.472**	2,097**	0.963	2,535***	0.993
IRT+PIP	1.84*	0.227**	0.658	0.563**	1,334	0.6**

mRNA expression levels are given as fold increase/decrease. *p<0.05, **p≤0.01 and ***p≤0.001. Fold increases/decreases and statistical analyses were performed using REST 2009 (Qiagen) software. IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine, Bad: Bcl-2-associated death promoter, Bax: Bcl-2-associated X protein, ATF4: Activating transcription factor 4, CHOP: C/EBP homologous protein, GRP78: Glucose-regulated protein 78

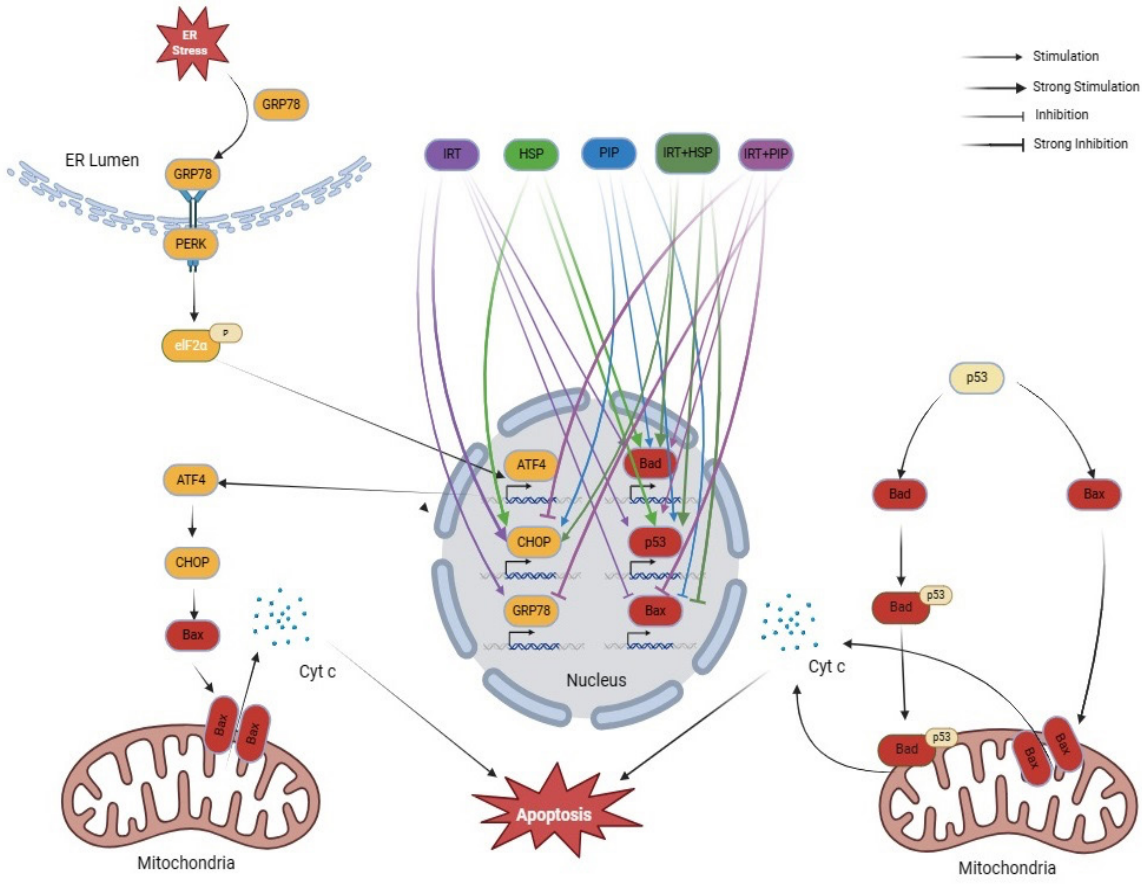

Figure 4. Effects of IRT, HSP and PIP on ER stress-mediated and p53-mediated apoptosis. It was created using Biorender
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

increased CHOP mRNA expression compared to the control group (p=0.001), as a result of the combination of IRT with PIP, CHOP mRNA expression level decreased to the level of the control group (p=0.123) (Figure 4). The combination of IRT with PIP resulted in the suppression of ATF4 and GRP78 mRNA expression levels, which are ER stress markers, in HCC compared to the control group (p=0.006 and p=0.009, respectively), and the CHOP mRNA expression level decreased to the level observed in the control group (p=0.123). There are data in the literature that contradict our findings (15). Although we showed in our study that PIP does not promote IRT on genes responsible for ER stress, the literature also shows that the use of PIP increases chemosensitivity to 5-fluorouracil in resistant colon cancer (16). Piperine is a potent inhibitor of permeability glycoprotein (P-glycoprotein 1) and cytochrome P450. P-glycoprotein 1 is also known as multidrug resistance protein 1 (MDR1) or CD243 and is an important protein that pumps many foreign substances out of the cell that may be harmful and is inhibited by PIP. Microsomal cytochrome P450 overproduction causes endoplasmic reticulum stress (17). In this regard, the observation that the ER stress markers were reduced in the groups where PIP, a cytochrome P450 inhibitor, was applied, compared to the control group, was found to be compatible with the literature.

It has been shown that HSP induces CHOP protein expression in ovarian cancer cells (18), and it inhibits the proliferation of HeLa cells through ER stress-mediated apoptosis (19). In this regard, although the mRNA expression levels of ATF4 and GRP78, which are ER stress markers, did not differ significantly from the control group in the HSP group (p=0.062 and p=0.774, respectively), the CHOP mRNA expression level was found to be higher than the control group. This result was found to be compatible with literature data (p=0.004).

Shrivastava et al. (20) suggested that PIP has anti-apoptotic properties, and we confirmed this finding in

our study. While IRT alone increased the mRNA expression levels of the *Bad* and *p53* genes (p=0.002 and p=0.007, respectively), HSP enhanced this effect more than IRT alone (p=0.008 and p=0.007, respectively). In the IRT+HSP group, both Bad and p53 mRNA expression levels were higher than in the IRT group (p=0.004 and p=0.005, respectively). Piperine, on the other hand, either eliminated or reduced the effect of IRT alone on apoptotic markers. Bax mRNA expression levels were lower than those of the control group in all groups except the HSP group (Figure 4). Although no anti-cancer effects were observed with the combined use of PIP and IRT in HCC in our study, some studies in the literature have reported anti-cancer effects when PIP is used alone. For example, it has been shown to induce apoptosis in oral cancer (21,22), gastric cancer (23), and breast cancer (24). Similarly, our study found that PIP increased apoptotic markers when used alone but reduced the apoptotic effects of IRT when used in combination. A literature review revealed no studies addressing the combination of IRT with PIP.

Study Limitations

This study has some limitations. Firstly, it focuses solely on mRNA expression levels without examining protein levels and activities, which limits the understanding of the functional effects of gene products on cellular processes. The cell culture conditions may not fully replicate the in vivo environment, and the specific characteristics of the cell line used may limit the generalizability of the results. Due to financial constraints, the scope of the study was kept narrow. Despite these methodological difficulties, this study is a preliminary study that can be cited in subsequent studies.

Conclusion

The findings of our study indicate significant differences between the use of IRT alone and its combination with HSP or PIP. The combination of IRT with HSP enhanced the expression of apoptosis-related genes, such as Bad, Bax, and p53, thereby supporting IRT's pro-apoptotic effects in HCC. In contrast, the combination of IRT with PIP showed a reduced effect on ER stress markers, including ATF4, CHOP, and GRP78, compared to IRT alone. This suggests that PIP may inhibit the anti-tumor activity of IRT by suppressing the expression of genes responsible for ER stress. These findings highlight the potential of HSP to augment IRT's therapeutic efficacy, while PIP appears to mitigate its anti-tumor effects.

Ethics

Ethics Committee Approval: This study was conducted using commercially obtained HepG2 cell lines. No patient samples or primary cell cultures were used. Therefore, ethical approval was not required.

Informed Consent: No patient samples or primary cell cultures were used.

Authorship Contributions

Concept: S.S., K.K.K., C.Y., S.C., Design: S.S., K.K.K., C.Y., S.C., Data Collection or Processing: S.S., K.K.K., C.Y., S.C., Analysis or Interpretation: S.S., K.K.K., C.Y., S.C., Literature Search: S.S., K.K.K., C.Y., S.C., Writing: S.S., K.K.K., C.Y., S.C.

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

References

1. Jiang Z, Wei Z, Chen J, Yang F, Jiang Y, Lv L. BZW2, CDT1 and IVD Act As Biomarkers for Predicting Hepatocellular Carcinoma. Curr Cancer Drug Targets. 2023;23:211-21.
2. Bruix J, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2021;53:1020-2.
3. Finn RS, Zhu AX, Farah W, et al. Therapies for advanced stage hepatocellular carcinoma with macrovascular invasion or metastatic disease: A systematic review and meta-analysis. Hepatology. 2020;72:414-27.
4. Chai Y, Liu JL, Zhang S, et al. The effective combination therapies with irinotecan for colorectal cancer. Front Pharmacol. 2024;5:1356708.
5. Šamec D, Karalija E, Šola I, Vujčić Bok V, Salopek-Sondi B. The Role of Polyphenols in Abiotic Stress Response: The Influence of Molecular Structure. Plants (Basel). 2021;10:118.
6. Pyrzynska K. Hesperidin: A Review on Extraction Methods, Stability and Biological Activities. Nutrients. 2022;14:2387.
7. Bahador A, Vaezi M. Novel formulations of hesperidin for antitumor, antimicrobial, and neuroprotective effects: A review. Micro Nano Bio Aspects. 2023;2:13-9.
8. Han EJ, Choi EY, Jeon SJ, et al. Piperine induces apoptosis and autophagy in hsc-3 human oral cancer cells by regulating PI3K signaling pathway. Int J Mol Sci. 2023;24:13949.
9. Liu S, Luo L, Zuo F, et al. Immunosuppression and apoptosis activation mediated by p53-Bcl2/Bax signaling pathway-The potential mechanism of goldfish (Carassius auratus Linnaeus) gill disease caused by Myxobolus ampullicapsulatus. Front Immunol. 2022;13:998975.
10. Sen S, Kasikci M. Upshot of Some Bioactive Compounds on Angiogenesis in Retinal Pigment Epithelial Cells. J Cell Mol Med. 2025;29:e70327.
11. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:e45.
12. Saurav S, Karfa S, Vu T, et al. Overcoming Irinotecan Resistance by Targeting Its Downstream Signaling Pathways in Colon Cancer. Cancers (Basel). 2024;16:3491.

13. Kumar S, Sherman MY. Resistance to TOP-1 Inhibitors: Good Old Drugs Still Can Surprise Us. Int J Mol Sci. 2023;24:7233.
14. Liu Q, Hua S, Wang X, Chen F, Gou S. The introduction of immunosuppressor (TDO inhibitor) significantly improved the efficacy of irinotecan in treating hepatocellular carcinoma. Cancer Immunol Immunother. 2021;70:497-508.
15. Wu C, Qian Y, Jiang J, Li D, Feng L. Piperine inhibits the proliferation of colorectal adenocarcinoma by regulating ARL3-mediated endoplasmic reticulum stress. Biomol Biomed. 2024;25:391-405.
16. Bhattacharjya D, Sivalingam N. Mechanism of 5-fluorouracil induced resistance and role of piperine and curcumin as chemo-sensitizers in colon cancer. Naunyn Schmiedebergs Arch Pharmacol. 2024;397:8445-75.
17. Memon TA, Sun L, Almestica-Roberts M, et al. Inhibition of TRPA1, Endoplasmic Reticulum Stress, Human Airway Epithelial Cell Damage, and Ectopic MUC5AC Expression by Vasaka (Adhatoda vasica; Malabar Nut) Tea. Pharmaceuticals (Basel). 2023;16:890.
18. Zhao J, Li Y, Gao J, De Y. Hesperidin inhibits ovarian cancer cell viability through endoplasmic reticulum stress signaling pathways. Oncol Lett. 2017;14:5569-74.
19. Wang Y, Yu H, Zhang J, et al. Hesperidin inhibits HeLa cell proliferation through apoptosis mediated by endoplasmic reticulum stress pathways and cell cycle arrest. BMC Cancer. 2015;15:682.
20. Shrivastava P, Vaibhav K, Tabassum R, et al. Anti-apoptotic and anti-inflammatory effect of piperine on 6-OHDA induced Parkinson's rat model. J Nutr Biochem. 2013;24:680-7.
21. Mitra S, Anand U, Jha NK, et al. Anticancer Applications and Pharmacological Properties of Piperidine and Piperine: A Comprehensive Review on Molecular Mechanisms and Therapeutic Perspectives. Front Pharmacol. 2022;12:772418.
22. Han EJ, Choi EY, Jeon SJ, et al. Piperine Induces Apoptosis and Autophagy in HSC-3 Human Oral Cancer Cells by Regulating PI3K Signaling Pathway. Int J Mol Sci. 2023;24:13949.
23. Guo L, Yang Y, Sheng Y, Wang J, Ruan S, Han C. Mechanism of piperine in affecting apoptosis and proliferation of gastric cancer cells via ROS-mitochondria-associated signalling pathway. J Cell Mol Med. 2021;25:9513-22.
24. Hakeem AN, El-Kersh DM, Hammam O, et al. Piperine enhances doxorubicin sensitivity in triple-negative breast cancer by targeting the PI3K/Akt/mTOR pathway and cancer stem cells. Sci Rep. 2024;14:18181.