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Molecular Mechanisms Underlying the Anticancer Activity of Chrysin Through p53 Tumor Suppressor in HepG2 Cell Lines

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Abstract

Chrysin, a natural flavonoid found in passionflower, honey, and propolis, is gaining attention for its antioxidant, anti-inflammatory, and anticancer properties. This study evaluates chrysin's anticancer efficacy against HepG2 liver cancer cells. We assessed its antioxidant potential using DPPH and nitric oxide scavenging assays, which revealed significant, concentration-dependent radical scavenging activity, emphasizing chrysin's strong antioxidant properties. These effects are likely to reduce oxidative stress, a factor that promotes cancer cell proliferation and survival. Cytotoxicity was measured with the MTT assay, and gene expression analysis through RT-qPCR showed that chrysin upregulated pro-apoptotic genes such as Bax, Caspase 3, and Caspase 9, while downregulating the anti-apoptotic gene Bcl-2. Notably, chrysin also increased the expression of the tumor suppressor gene p53, essential for cell cycle regulation and apoptosis in response to stress and DNA damage. Molecular docking studies were performed to investigate chrysin's interactions with key apoptotic proteins. The docking results showed strong binding affinities between chrysin and Bax, Bcl-2, Caspase 3, Caspase 9, and p53. Particularly high binding affinities with Caspase 9 and p53 suggest that chrysin may effectively trigger the intrinsic apoptotic pathway, leading to cancer cell death. The interaction with p53 is significant as it may stabilize and activate p53, enhancing the transcription of pro-apoptotic genes. These findings highlight chrysin's potential as a therapeutic agent for liver cancer, primarily through the p53-mediated apoptotic pathway. While these in vitro results are promising, further in vivo studies and clinical trials are necessary to confirm chrysin's efficacy and safety in a clinical setting.

Keywords: *Apoptosis, Antioxidant Activity, Chrysin, Gene Expression Analysis, Hepatocellular Carcinoma, Novel Methods.*

Introduction

Cancer remains a major global health challenge, driving an urgent need for the discovery and development of novel therapeutic agents. Hepatocellular carcinoma (HCC), the most prevalent form of primary liver cancer, presents significant treatment difficulties due to its typically late diagnosis, aggressive progression, and resistance to conventional therapies. As a result, research has increasingly focused on natural compounds with potential anticancer properties [1]. Among these, chrysin naturally occurring flavonoid found in honey, propolis, and various plants has emerged as a compound of interest due to its

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broad spectrum of biological activities, including notable anticancer effects.

HepG2 cells are a prominent human liver cancer cell line extensively utilized in biomedical research. Originally derived from the liver tissue of a 15-year-old male with hepatocellular carcinoma in 1975, these cells are valued for their hepatocyte-like properties, which make them an indispensable in vitro model for studying various aspects of liver function, disease, and pharmacology. Their ability to perform many liver-specific activities, such as protein synthesis and drug metabolism, renders them particularly useful in investigating liver diseases, evaluating the hepatotoxicity of new drugs, and understanding liver-specific metabolic pathways [2-4]. The significance of HepG2 cells extends across multiple research domains. In the realm of drug development, they serve as a critical tool for screening potential hepatotoxic agents and studying the metabolism of pharmaceuticals. Their consistent expression of liver-specific genes and enzymes allows for a detailed examination of gene regulation and expression in hepatic contexts [5, 6]. Additionally, HepG2 cells contribute to the understanding of liver cancer mechanisms and the development of therapeutic strategies, given their origin from hepatocellular carcinoma. Despite certain limitations, such as their cancerous origin which may not perfectly reflect normal liver physiology, HepG2 cells offer numerous advantages including ease of culture, stable genetic characteristics, and reproducible results. These attributes ensure their ongoing relevance and utility in scientific research, making HepG2 cells a cornerstone in the study of liver biology and related fields.

Chrysin (5,7-dihydroxyflavone) is well-documented for its anti-inflammatory, antioxidant, and anticancer properties. Its anticancer effects are particularly compelling, as chrysin has been shown to influence multiple cellular processes that contribute to tumorigenesis. These processes include the modulation of cell proliferation, induction of apoptosis, and inhibition of metastasis. The specific molecular mechanisms underlying these effects, however, require further elucidation to fully understand and harness chrysin's therapeutic potential [7]. One promising area of research focuses on the interaction between chrysin and the tumor suppressor protein p53, which plays a central role in regulating the cell cycle and apoptosis.

The p53 protein, often dubbed the "guardian of the genome," is crucial for maintaining cellular integrity by preventing the propagation of DNA-damaged cells. It achieves this through the activation of pathways that lead to cell cycle arrest, DNA repair, or apoptosis in response to genomic stress. In many cancers, including HCC, p53 is frequently mutated or functionally impaired, resulting in uncontrolled cell division and tumor growth. Restoring or enhancing the function of p53 in cancer cells has therefore become a strategic target in cancer therapy. Chrysin's potential to modulate the p53 pathway positions it as a promising candidate for therapeutic development, particularly in cancers where p53 is compromised. In HepG2 cells, a human hepatocellular carcinoma cell line, chrysin has demonstrated the ability to induce apoptosis and cell cycle arrest, primarily through mechanisms involving the p53 pathway. This introduction delves into the molecular mechanisms by which chrysin activates and modulates the p53 tumor suppressor pathway in HepG2 cells, highlighting its role in promoting cell death and inhibiting cell proliferation. By elucidating these pathways, this study aims to provide a comprehensive understanding of chrysin's anticancer mechanisms, paving the way for its potential application as a therapeutic agent in the treatment of hepatocellular carcinoma and other malignancies characterized by p53 dysfunction.

Materials and Method

Chemicals and Reagents

Chrysin purchased from sigma (C80105), Dulbecco's Modified Eagle's Medium (DMEM), antibiotics (streptomycin and penicillin solution), phosphate-buffered saline (PBS), trypsin-EDTA, and dimethyl sulfoxide (DMSO) were procured from HIMEDIA, Mumbai, India. The MTT reagent was obtained from SRL. The drug troxerutin was dissolved in DMSO to prepare initial stock solutions at a concentration of 5mM, which were then stored at -20ºC.

DPPH and Nitric Oxide Scavenging Activity

The assessment of DPPH activity followed the procedure outlined by Hatano et al. (1989) [8]. A mixture of 1.0 ml of DPPH solution and 1.0 ml of extract (concentrations ranging from 0.1 to 0.5 mg/ml) was prepared, and its activity was measured at a wavelength of 517 nm. Ascorbic acid at equivalent concentrations was used as the standard. The scavenging of the nitric oxide radical was evaluated using the standard method. To 0.5 mL of chrysin at concentrations of 100–500 µL, 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate-buffered saline (pH 7.4) was added and incubated at 25°C for 150 minutes. Then, 0.5 mL of the mixture was combined with 1.0 mL of sulfanilic acid reagent, followed by the addition of 1.0 mL of naphthyl ethylenediamine dihydrochloride (0.1% w/v). The activity was measured at 540 nm after a 30-minute incubation.

Cell lines and Subculture

The HepG2 cells used in this study were purchased from the NCCS cell repository in Pune, India. For subculturing, the tissue culture plates containing HepG2 cells were inspected under a microscope to assess the degree of confluency and ensure the absence of bacterial and fungal contaminants. Upon reaching 80% confluency, the medium was aspirated from the tissue culture plates, and the cells were rinsed with 1 ml of 1x PBS, followed by another aspiration. Then, 1 ml of trypsin-EDTA solution was added to the washed cell monolayer, and the plate was gently rotated to ensure the trypsin-EDTA covered the entire monolayer. The plate was then incubated for 1 minute at 37°C in a 5% CO_2 incubator to allow the adherent cells to detach completely from the surface of the plate. The cells were observed under a microscope to confirm their dissociation from each other. After complete detachment, 3 ml of complete medium was added to the culture vessel to neutralize the trypsin-EDTA. The detached cells were then centrifuged at 1500 rpm for 2 minutes. The resulting pellet was collected and resuspended in 1 ml of complete medium. The cells were subsequently seeded and routinely maintained in sterile tissue culture plates with a complete medium containing 10% fetal bovine serum and antibiotics (penicillin and streptomycin) at 37°C in a 5% CO_2 incubator.

Cell Viability Assay

Once the cells reached 80% confluency in the tissue culture plates, they were harvested and collected. HepG2 cells were seeded at a density of 2000 cells per well into a 96-well microplate, in triplicate. The 96-well plate was then incubated overnight at 37°C with 5% CO_2 to allow cell growth. Following incubation, the cells were treated with increasing concentrations of troxerutin (0-200 µM) and incubated for an additional 48 hours in the CO_2 incubator. Cell viability was assessed using the MTT assay. Cells were incubated with 5 mg/ml MTT for 2 hours at 37°C in a 5% CO_2 incubator. After incubation, the MTT solution was discarded, and 100 µl of dimethyl sulfoxide (DMSO) was added to each well. Absorbance was measured at 570 nm using a microplate reader. The number of viable cells was expressed as a percentage of the control cells cultured in a serum-free medium, with cell viability in the untreated control medium represented as 100%.

Gene Expression Analysis

Total RNA was isolated from control and experimental samples using a TRIR (Total RNA Isolation Reagent) kit. Briefly, the cells were homogenized with 1 ml of TRIR, and the homogenate was immediately transferred to a microfuge tube and stored at -80°C for 60 minutes to allow complete dissociation of nucleoprotein complexes. Subsequently, 0.2 ml of chloroform was added to the homogenate, which was then vortexed for 1 minute and placed on ice at 4°C for 5 minutes. The mixture was centrifuged at 12,000xg for 15 minutes at 4°C. The aqueous phase was carefully transferred to a new microfuge tube, and an equal volume of isopropanol was added, followed by vortexing for 15 seconds and incubation on ice at 4°C for 10 minutes. The samples were then centrifuged at 12,000xg for 10 minutes at 4°C. The supernatant was discarded, and the RNA pellet was washed with 1 ml of 75% ethanol by vortexing and centrifugation at 7,500xg for 5 minutes at 4°C. After removing the supernatant, the RNA pellets were resuspended in 50 µl of autoclaved Milli-Q water and dissolved by heating in a water bath at 60°C for 10 minutes. Total RNA was reverse transcribed into cDNA using a commercial kit from Takara, as per the manufacturer's instructions. This cDNA was further used for the RT-PCR analysis. In the RT-PCR reaction mixture containing SyBr green master mix, the sequences of the primers were, Bax: Forward primer: 5'-ATGGACGGGTCCGGGGAG-3', Reverse primer: 5'-TCAGCCCATCTTCTTCCAGATGG-3', Bcl-2: Forward primer: 5'-ATGGTGGGGTCATGTGTGTGG-3', Reverse primer: 5'-TCAGGCACTTGTGGCCCAG-3', Caspase 3: Forward primer: 5'-ATGGACGGGTCCGGGGAG-3', Reverse primer: 5'-TCAGCCCATCTTCTTCCAGATGG-3', Caspase 9: Forward primer: 5'-ATGGACGGGTCCGGGGAG-3', Reverse primer: 5'-TCAGCCCATCTTCTTCCAGATGG-3', p53: Forward primer: 5'-ATGGACGGGTCCGGGGAG-3', Reverse primer: 5'-TCAGCCCATCTTCTTCCAGATGG-3'.

Protein Preparation

Using the Protein Data Bank (PDB), three-dimensional structures of mTOR-mediated signaling targets (Bax, Bcl-2, Caspase 3, 9, and p53) were obtained. The .pdb files were processed through the "Build/Check/Repair Model" and "Prepare the PDB File for Docking Programs" modules, where minor adjustments were made to restore missing side chains. Additionally, the positions of water molecules were corrected, symmetrical alignment was ensured, and hydrogen atoms were added. For the preparation of pdbqt files, AutodockTools was used to process only chain A of the repaired .pdb files. Water molecules and irregular residues were removed, retaining only polar hydrogens, and Kollman charges were calculated for the protein atoms using ADT.

Ligand and Docking Preparation

Chrysin, a phytochemical compound, was visualized using ChemSketch-12.01 software, and its geometric representation was optimized using the Austin Model 1. The optimized structure was then imported into AutodockTools (ADT) for the creation of a .pdbqt file for docking studies with AutoDock4. AutoDock4 was employed for the docking studies, utilizing the Lamarckian Genetic Algorithm with Local Search (GALS) as the search engine, with a total of 100 runs. The docking region of interest, used by AutoDock4 for docking runs and by AutoGrid4 for affinity grid map preparation, was defined to encompass the entire catalytic binding site. This was achieved using a grid size of 40 x 40 x 40 points with a grid spacing of 0.325 Å, centered on the grid box. Cluster analysis of the docked results was performed using a root mean square

(RMS) tolerance of 2.0 Å. Finally, the most energetically favorable cluster poses were evaluated using Biovia Discovery Studio 2021.

Software Used for Molecular Docking

Ligand preparation was accomplished using ACD/ChemSketch 12.01 (Advanced Chemistry Development, Inc). Geometrical representations were optimized with PubChem. Protein preparation was carried out using the Wizard feature of AutoDock Tools 1.5.4. Molecular docking calculations were completed using AutoDock Tools and MGL Tools 1.5.4 packages from The Scripps Research Institute, Molecular Graphics Laboratory, CA, USA.

Statistical Analysis

The acquired data underwent a comprehensive analysis using the one-way analysis of variance (ANOVA) method. For post hoc comparisons, the Multiple Range tests by Duncan were selected. Subsequently, a significance threshold of $P < 0.05$ was employed for determining statistical significance.

Results

Invitro Assay Analysis of Chrysin

The antioxidant activity of chrysin was evaluated using the DPPH radical scavenging assay. Chrysin demonstrated significant DPPH radical scavenging activity in a dose-dependent manner. At the highest concentration tested, chrysin exhibited a scavenging activity comparable to that of ascorbic acid, the standard antioxidant used in this assay (Figure 1A & Table 1). Chrysin's ability to scavenge nitric oxide radicals was performed. The results showed that chrysin effectively scavenged NO radicals in a concentration-dependent manner. At concentrations ranging from 12.5 to 200 µM, chrysin significantly reduced the formation of NO radicals (Figure 1B & Table 1).

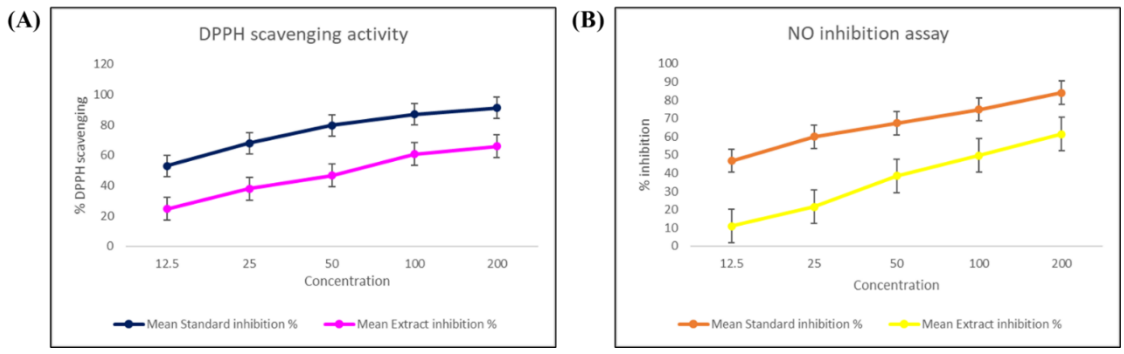

Figure 1: DPPH Scavenging Activity and NO Activity of Chrysin.

Table 1. Represents the DPPH and NO Scavenging Activity (% of inhibition). Values are Expressed in Mean ± SD of 3 Replication

Concentration (µg/ml)	Mean Standard Inhibition %	Mean of compound inhibition %	Mean Standard Inhibition %	Mean of compound inhibition %
	DPPH		**NO**	
12.5	52.87	24.56	46.76	10.89
25	67.89	37.87	59.89	21.45
50	79.56	46.54	67.34	38.56
100	86.9	60.67	74.9	49.56
200	91.23	65.87	84.09	61.43

Cytotoxicity Activity of Chrysin

Chrysin's impact on HepG2 cell viability was evaluated via an MTT assay, revealing a dose-dependent decline in viability (Figure 2). At 5 µg/ml, viability slightly decreased compared to the control, suggesting minimal cytotoxicity at low doses. As chrysin concentrations rose to 10 µg/ml and 20 µg/ml, viability dropped to approximately 80% and 70%, respectively. Higher concentrations (40 µg/ml, 80 µg/ml, and 160 µg/ml) induced significant cytotoxic effects, reducing viability to about 50%, 30%, and below 10%, respectively. At 160 µg/ml, chrysin demonstrated maximum cytotoxicity, nearly eliminating cell viability. These findings underscore chrysin's ability to dose-dependently inhibit HepG2 cell proliferation, suggesting its potential as an anticancer agent effective against hepatocellular carcinoma.

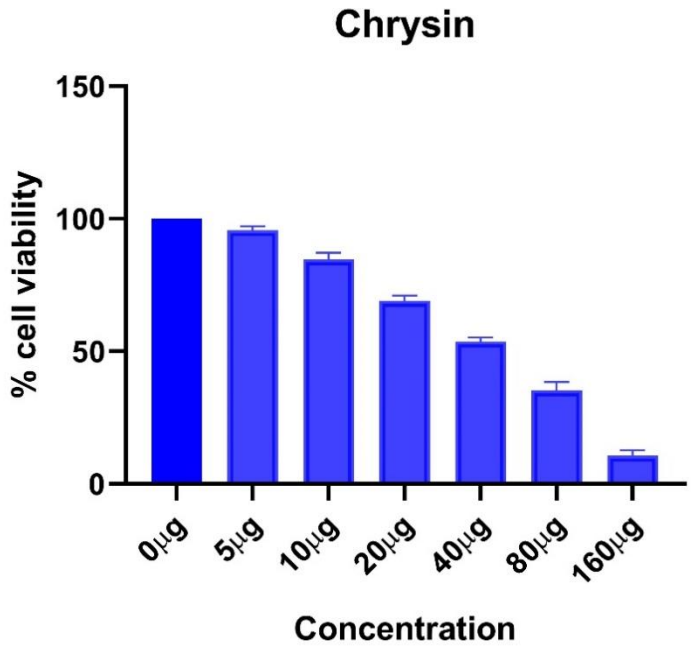

Figure 2: Cytotoxic Effect of Chrysin in HepG2 cells.

The gene expression analysis was conducted to evaluate the impact of chrysin on key apoptosis-related genes in HepG2 cells. After treating the cells with chrysin, the mRNA levels of Bax, Bcl-2, Caspase 3, Caspase 9, and p53 were quantified using RT-qPCR (Figure 3). The results indicated significant changes in the expression of these genes, signifying the activation of apoptotic pathways. The pro-apoptotic gene Bax was markedly upregulated in chrysin-treated HepG2 cells compared to untreated controls, suggesting that chrysin enhances Bax expression, which is involved in mitochondrial-mediated apoptosis. Conversely, the anti-apoptotic gene Bcl-2 was significantly downregulated following chrysin treatment, indicating that chrysin disrupts the balance between pro- and anti-apoptotic signals in favor of apoptosis. Both caspase 3 and 9, critical executors of apoptosis, exhibited increased mRNA levels in response to chrysin treatment. This upregulation suggests that chrysin activates the caspase cascade, leading to programmed cell death. Furthermore, the tumor suppressor gene p53 was notably upregulated in chrysin-treated cells, which is crucial as p53 is a key regulator of the apoptotic response to cellular stress and DNA damage.

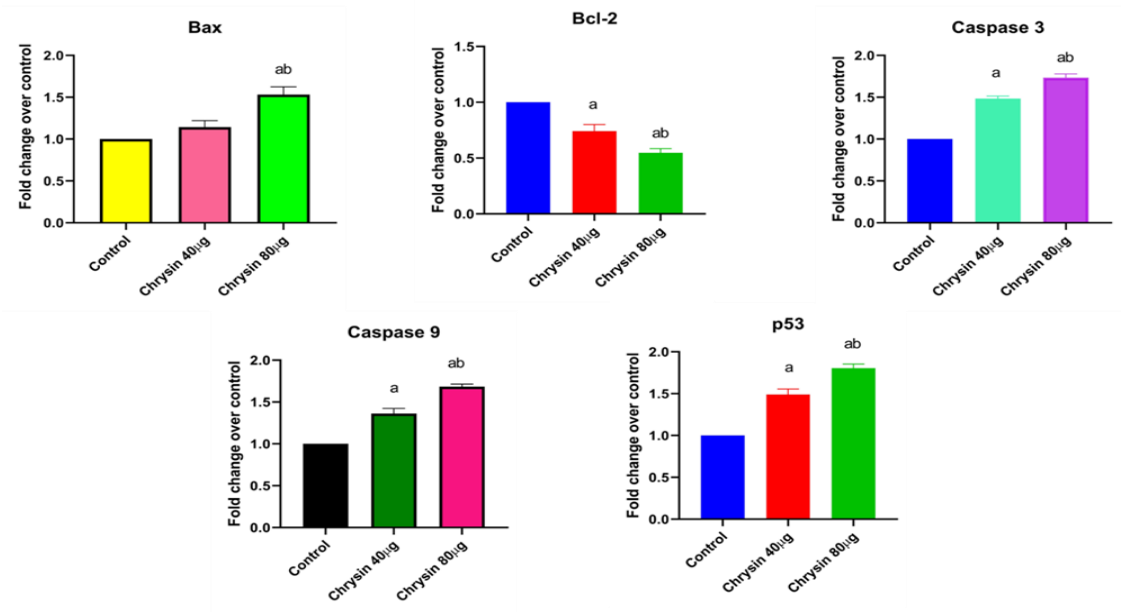

Figure 3: Effect of Chrysin in HepG2 on Bax, Bcl-2, Caspase 3, Caspase 9, and p53 Expression.

Binding Interactions of Chrysin with Bax, Bcl-2, Caspase 3, Caspase 9, and p53 Targets

Molecular docking studies revealed that chrysin interacts with various apoptotic proteins, providing insights into its anticancer effects (Figure 4 and Table 2). Binding energies with Bax (-7.8 kcal/mol), Bcl-2 (-7.5 kcal/mol), Caspase 3 (-7.6 kcal/mol), Caspase 9 (-8.5 kcal/mol), and p53 (-7.4 kcal/mol) were calculated. Chrysin's strong interaction with Bax suggests enhanced pro-apoptotic activity, while its interaction with Bcl-2 indicates inhibition of anti-apoptotic functions. The binding with Caspase 3 supports its activation, and the particularly strong interaction with Caspase 9 suggests effective initiation of the caspase cascade. Interaction with p53 implies stabilization and activation, promoting the transcription of pro-apoptotic genes.

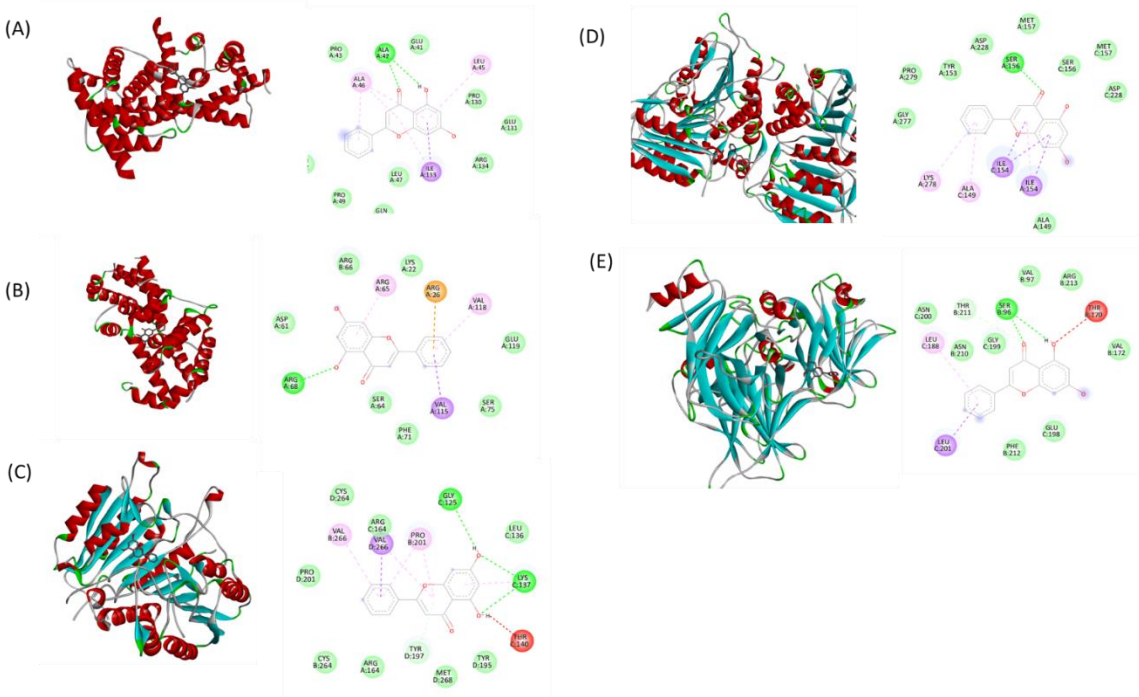

Figure 4: 3D, 2D Structure of Docking Interactions of Chrysin with Target Proteins.

Table 2: Binding interactions of chrysin with Bax, Bcl-2, Caspase 3, Caspase 9, and p53.

S.No	Ligand	Protein Target	Binding energy (kcal/mol)
1	Chrysin (5281607)	Bax	-7.8
2		Bcl-2	-7.5
3		Caspase-3	-7.6
4		Caspase-9	-8.5
5		p53	-7.4

Discussion

HepG2 is a well-established human hepatocellular carcinoma (HCC) cell line, widely used in scientific research to study liver cancer. Derived from the liver tissue of a 15-year-old Caucasian male with a well-differentiated HCC, HepG2 cells provide a valuable model for exploring the molecular and cellular mechanisms underlying liver cancer, testing the efficacy of anticancer agents, and investigating liver-specific functions [2]. HepG2 cells are extensively utilized due to their robust growth characteristics and ability to retain many of the differentiated functions of normal hepatocytes, including the production of plasma proteins and the capacity for biotransformation. These features make HepG2 an ideal in vitro model for studying not only hepatocarcinogenesis but also drug metabolism and hepatotoxicity. In the context of this study, HepG2 cells were instrumental in assessing the anticancer activity of chrysin. The use of this cell line allowed for a detailed examination of chrysin's cytotoxic effects, mechanisms of action, and impact on gene expression related to apoptosis. Specifically, the ability of chrysin to reduce cell viability in HepG2 cells, as demonstrated by the MTT assay, underscores its potential as a therapeutic agent for liver cancer [9-14]. Chrysin, a natural flavonoid predominantly found in passionflower, honey, and propolis, has garnered significant attention due to its multifaceted biological activities. Known for its potent antioxidant, anti-inflammatory, and anticancer properties, chrysin has been extensively studied in various cancer models.

The present study investigates the molecular mechanisms underlying the anticancer activity of chrysin, with a particular focus on its interaction with the p53 tumor suppressor in HepG2 cell lines. Through a series of in vitro assays, including DPPH and nitric oxide scavenging, cytotoxicity testing, mRNA gene expression analysis, and molecular docking studies, we have elucidated the potential pathways through which chrysin exerts its effects. The antioxidant potential of chrysin was evaluated using DPPH and nitric oxide scavenging assays. Chrysin demonstrated significant radical scavenging activity in both assays, suggesting that its antioxidant properties may contribute to its anticancer effects. The ability of chrysin to neutralize free radicals could reduce oxidative stress within the cancer cells, potentially leading to decreased cellular proliferation and increased apoptosis. The cytotoxic effects of chrysin on HepG2 cells were assessed using the MTT assay. Chrysin exhibited a dose-dependent reduction in cell

viability, indicating its potential as a cytotoxic agent against liver cancer cells. The decrease in absorbance at 570 nm correlates with reduced cell viability, suggesting that chrysin effectively inhibits HepG2 cell proliferation.

To further elucidate the mechanism of action of chrysin, the expression levels of key apoptosis-related genes (Bax, Bcl-2, Caspase 3, Caspase 9, and p53) were analyzed. The results revealed that chrysin treatment led to an upregulation of pro-apoptotic genes (Bax, Caspase 3, and Caspase 9) and a downregulation of the anti-apoptotic gene Bcl-2. Notably, the expression of the tumor suppressor gene p53 was significantly increased, indicating that p53 may play a critical role in chrysin-induced apoptosis. Molecular docking studies were performed to understand the binding interactions of chrysin with Bax, Bcl-2, Caspase 3, Caspase 9, and p53. The binding energies for chrysin with these proteins are as follows: Bax: -7.8, Bcl-2: -7.5, Caspase 3: -7.6, Caspase 9: -8.5, p53: -7.4 kcal/mol. The docking results suggest that chrysin has a strong binding affinity for all these targets, particularly Caspase 9, indicating a robust interaction. The interaction with p53 is of particular interest as it may elucidate the mechanism through which chrysin exerts its anticancer effects.

The upregulation of p53 and its interaction with chrysin suggest that the anticancer activity of chrysin is mediated through the activation of the p53 pathway. p53 is a crucial tumor suppressor that regulates the cell cycle and induces apoptosis in response to cellular stress [15-20]. The activation of p53 leads to the transcription of various downstream targets, including pro-apoptotic genes like Bax and Caspases, which promote apoptosis. The increased expression of Bax, Caspase 3, and Caspase 9, along with the decreased expression of Bcl-2, supports this hypothesis. Chrysin's binding to p53 and other apoptotic proteins facilitates the activation of the intrinsic apoptotic pathway, resulting in mitochondrial dysfunction and activation of caspases, which ultimately leads to cell death. This mechanism is further supported by the binding interactions observed in the docking studies, where chrysin exhibited significant binding affinities to these proteins.

Conclusion

This study provides comprehensive insights into the molecular mechanisms underlying the anticancer activity of chrysin in HepG2 cell lines. The *in vitro* assays confirmed chrysin's antioxidant and cytotoxic properties. Gene expression analysis and molecular docking studies suggest that chrysin induces apoptosis through the p53-mediated pathway. Chrysin's interaction with p53 and other apoptotic proteins like Bax, Caspase 3, and 9 highlights its potential as a promising therapeutic agent in the treatment of liver cancer. Further, *in vivo* studies and clinical trials are warranted to validate these findings and explore the therapeutic potential of chrysin in cancer treatment.

Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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References

[1]. Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Zucman-Rossi, J., & Finn, R. S., 2021, Hepatocellular carcinoma. *Nature Reviews. Disease*

Primers, *7*(1), 6. https://doi.org/10.1038/s41572-020-00240-3

[2]. Khemlina, G., Ikeda, S., & Kurzrock, R., 2017, The biology of Hepatocellular carcinoma: implications for genomic and immune therapies. *Molecular Cancer*, *16*(1), 149. https://doi.org/10.1186/s12943-017-0712-x

[3]. Hong, W., Zhang, Y., Wang, S., Zheng, D., Hsu, S., Zhou, J., Fan, J., Zeng, Z., Wang, N., Ding, Z., Yu, M., Gao, Q., & Du, S., 2024, Deciphering the immune modulation through deep transcriptomic profiling and therapeutic implications of DNA damage repair pattern in hepatocellular carcinoma. *Cancer Letters*, *582*, 216594. https://doi.org/10.1016/j.canlet.2023.216594

[4]. Zheng, J., Chen, J., Wang, S., Yang, D., & Zhou, P., 2024, Genomic and immune landscape in hepatocellular carcinoma: Implications for personalized therapeutics. *Environmental Toxicology*, *39*(3), 1601–1616. https://doi.org/10.1002/tox.24062

[5]. Wang, Y., & Chen, H., 2023, Protein glycosylation alterations in hepatocellular carcinoma: function and clinical implications. *Oncogene*, *42*(24), 1970–1979. https://doi.org/10.1038/s41388-023-02702-w

[6]. Mo, Z., Liu, D., Rong, D., & Zhang, S., 2021, Hypoxic Characteristic in the Immunosuppressive Microenvironment of Hepatocellular Carcinoma. *Frontiers in Immunology*, *12*, 611058. https://doi.org/10.3389/fimmu.2021.611058

[7]. Mani, R., & Natesan, V., 2018, Chrysin: Sources, beneficial pharmacological activities, and molecular mechanism of action. *Phytochemistry*, *145*, 187-196.

[8]. Hatano, T., Yasuhara, T., Yoshihara, R., Agata, I., Noro, T., & OKUDA, T., 1990, Effects of interaction of tannins with co-existing substances. VII.: inhibitory effects of tannins and related polyphenols on xanthine oxidase. *Chemical and Pharmaceutical Bulletin*, *38*(5), 1224-1229.

[9]. Jayaraman, S., Natararaj, S., & Veeraraghavan, V. P., 2024, Hesperidin inhibits oral cancer cell growth via apoptosis and inflammatory signaling-mediated mechanisms: Evidence from in vitro and in silico analyses. *Cureus*, *16*(2).

[10]. Jayaraman, S., Natarajan, S. R., Veeraraghavan, V. P., & Jasmine, S., 2023, Unveiling the anti-cancer mechanisms of calotropin: Insights into cell growth inhibition, cell cycle arrest, and metabolic regulation in human oral squamous carcinoma cells (HSC-3). *Journal of Oral Biology and Craniofacial Research*, *13*(6), 704-713.

[11]. Jayaraman, S., Veeraraghavan, V. P., Natarajan, S. R., & Jasmine, S., 2024, Exploring the therapeutic potential of curcumin in oral squamous cell carcinoma (HSC-3 cells): Molecular insights into hypoxia-mediated angiogenesis. *Pathology-Research and Practice*, *254*, 155130.

[12]. Roy, J. R., Janaki, C. S., Jayaraman, S., Periyasamy, V., Balaji, T., Vijayamalathi, M., & Veeraraghavan, V. P., 2022, Carica papaya reduces muscle insulin resistance via IR/GLUT4 mediated signaling mechanisms in high fat diet and streptozotocin-induced type-2 diabetic rats. *Antioxidants*, *11*(10), 2081.

[13]. Roy, J. R., Janaki, C. S., Jayaraman, S., Periyasamy, V., Balaji, T., Vijayamalathi, M., & Prasad, M., 2023, Carica papaya reduces high fat diet and streptozotocin-induced development of inflammation in adipocyte via IL-1β/IL-6/TNF-α mediated signaling mechanisms in type-2 diabetic rats. *Current Issues in Molecular Biology*, *45*(2), 852.

[14]. Deenadayalan, A., Subramanian, V., Paramasivan, V., Veeraraghavan, V. P., Rengasamy, G., Coiambatore Sadagopan, J., & Jayaraman, S., 2021, Stevioside attenuates insulin resistance in skeletal muscle by facilitating IR/IRS-1/Akt/GLUT 4 signaling pathways: An in vivo and in silico approach. *Molecules*, *26*(24), 7689.

[15]. Narasimhan, A., Sampath, S., Jayaraman, S., & Karundevi, B., 2013, Estradiol favors

glucose oxidation in gastrocnemius muscle through modulation of insulin signaling molecules in adult female rats. *Endocrine Research, 38*(4), 251-262.

[16]. Roy, J. R., Janaki, C. S., Jayaraman, S., Periyasamy, V., Balaji, T., Vijayamalathi, M., & Veeraraghavan, V. P., 2022, Effect of Carica papaya on IRS-1/Akt signaling mechanisms in High-Fat-Diet–Streptozotocin-Induced type 2 diabetic experimental rats: A mechanistic approach. *Nutrients, 14*(19), 4181.

[17]. Perumal, S., Langeshwaran, K., Selvaraj, J., Ponnulakshmi, R., Shyamaladevi, B., & Balasubramanian, M. P., 2018, Effect of diosmin on apoptotic signaling molecules in N-nitrosodiethylamine-induced hepatocellular carcinoma in experimental rats. *Molecular and Cellular Biochemistry, 449,* 27-37.

[18]. Devarajan, N., Jayaraman, S., Mahendra, J., Venkatratnam, P., Rajagopal, P., Palaniappan, H., & Ganesan, S. K., 2021, Berberine—A potent chemosensitizer and chemoprotector to conventional cancer therapies. *Phytotherapy Research, 35*(6), 3059-3077.

[19]. Selvaraj, J., Sathish, S., Mayilvanan, C., & Balasubramanian, K., 2013, Excess aldosterone-induced changes in insulin signaling molecules and glucose oxidation in gastrocnemius muscle of adult male rat. *Molecular and Cellular Biochemistry, 372,* 113-126.

[20]. Venditto, V. J., & Simanek, E. E., 2010, Cancer therapies utilizing the camptothecins: A review of the in vivo literature. *Molecular Pharmaceutics*, 7(2), 307–349. https://doi.org/10.1021/mp900243b