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Effect of Hypericum Perforatum Extract on cell viability and p53 localization in HepG2 cells

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Abstract

Aim: This study aimed to investigate the effect of Hypericum perforatum extract on cell viability and p53 localization in HepG2 cells.
Materials and methods: HepG2 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum and antibiotics. The treatment group was exposed to 0.8% Hypericum perforatum extract for 24 hours, while untreated cells served as the control group. Cell viability was evaluated using the CCK-8 assay, and absorbance was measured with a microplate reader. The intracellular localization of p53 protein was assessed by immunofluorescence staining and analyzed using confocal microscopy. Statistical analyses were performed using GraphPad Prism 10.0, and $p < 0.05$ was considered statistically significant.
Results: Treatment with 0.8% Hypericum perforatum extract significantly reduced CCK-8-based cell viability in HepG2 cells compared with the control group. Although total p53 fluorescence intensity did not show a marked difference between the groups, confocal microscopy indicated a redistribution of p53 signal from the cytoplasmic region toward the nucleus in HP-treated cells.
Conclusion: Hypericum perforatum extract reduced cell viability in HepG2 cells and was associated with altered subcellular localization of p53. These findings suggest that HP extract may induce a cellular stress response in HepG2 cells. However, further dose-response studies and additional molecular analyses are required to determine whether this effect is mediated through p53-dependent apoptotic pathways.
Keywords: Hypericum perforatum, HepG2, cell viability, p53, liver cancer model, CCK-8

Hypericum Perforatum Ekstraktının HepG2 hücrelerinde hücre canlılığı ve p53 lokalizasyonu üzerine etkisi

Öz

Amaç: Bu çalışmada, Hypericum perforatum (HP) ekstraktının HepG2 karaciğer kanseri hücrelerinde hücre canlılığı ve p53 proteininin hücre içi lokalizasyonu üzerindeki etkilerinin araştırılması amaçlandı.
Gereç ve Yöntem: HepG2 hücreleri standart kültür koşullarında çoğaltıldı. Deney grubuna 24 saat süreyle %0,8 HP ekstraktı uygulanırken kontrol grubuna herhangi bir uygulama yapılmadı. Hücre canlılığı CCK-8 testi ile değerlendirildi. p53 proteininin hücre içi dağılımı immünfloresan boyama ve konfokal mikroskopi ile incelendi. İstatistiksel analizlerde GraphPad Prism 10.0 kullanıldı ve $p<0,05$ anlamlı kabul edildi.
Bulgular: %0,8 HP ekstraktı uygulanan HepG2 hücrelerinde kontrol grubuna kıyasla hücre canlılığında anlamlı azalma saptandı ($p<0,05$). Toplam p53 immünfloresan yoğunluğu açısından gruplar arasında belirgin fark gözlenmezken, konfokal mikroskopi analizleri HP uygulanan hücrelerde p53'ün sitoplazmadan çekirdeğe doğru yeniden dağılım gösterdiğini ortaya koydu.
Sonuç: Hypericum perforatum ekstraktı HepG2 hücrelerinde hücre canlılığını azaltmış ve p53'ün hücre içi lokalizasyonunda değişikliğe neden olmuştur. Bulgular, HP ekstraktının hücresel stres yanıtını tetikleyebileceğini düşündürmektedir. Bununla birlikte, etkinin p53 aracılı apoptotik mekanizmalar üzerinden gerçekleşip gerçekleşmediğinin ortaya konulabilmesi için doz-cevap çalışmaları ve ileri moleküler analizlere ihtiyaç vardır.
Anahtar Kelimeler: Hypericum perforatum, HepG2, hücre canlılığı, p53, karaciğer kanseri, CCK-8

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Introduction

Liver cancer is the 3rd leading cause of cancer-related deaths (1,2). It is estimated that it will continue to be a global health problem with an estimated incidence of more than 1 million cases by 2025. Hepatocellular carcinoma (HCC) is the most common form of liver cancer, accounting for approximately 90% of cases (3).

Although hepatectomy is currently considered the most effective treatment for HCC, the recurrence rate of tumors within five years after resection is as high as 70%. Identification of molecules that cause HCC may provide improvements in the diagnosis and treatment of HCC (4).

Human tissue-derived cancer cell lines have been genetically and/or epigenetically characterized to identify specific cellular pathways and altered proteins in critical genes in cancer (5). Cell lines are known for their indefinite lifespan, stable phenotypes, high division rates, and ease of use. However, their main limitation is the lower expression of some metabolic activities compared to hepatocytes (6). Human hepatoblastoma-derived cell line (HepG2) has been widely used in liver and liver cancer studies to investigate hepatotoxicity, hepatic metabolism and hepatic absorption/excretion of candidate drugs (7-9). HepG2 cells are non-tumorigenic and highly proliferative cells that have been successfully grown in large-scale culture systems (10). They are the cells that most resemble tumor cells in hepatoblastoma, but they also retain the characteristic features of normal hepatocytes (11,12).

As a transcription factor, p53 regulates the expression of multiple target genes and is crucial for controlling the cell cycle, apoptosis and genomic stability, all of these functions contribute to tumor suppression (13). Most HCCs exhibit abnormalities in the p53-mediated apoptotic pathway. High in vivo p53 expression in HCC, may clinically seem as induced apoptosis and inhibitted tumor cell growth through various biological pathways. Also It's observed that p53 elevation can improve the susceptibility of human HCC cells to the anticancer drugs (14).

Hypericum perforatum (HP), belonging to the Hypericaceae family, is commonly known as St. John's Wort and is a perennial herbaceous plant native to Europe and Asia (15). There are many pharmacological studies on the anti-inflammatory, antidepressant, antimicrobial and antiviral activities of HP extracts. (16).

The studies for plant oils, extracts and active compounds with anticancer activity seems to be a vital strategy for the development of new anticancer agents with different modes of action or lower toxic effects. HP has been widely used among the public to treat various disorders such as depression, peptic ulcers and wounds. There are also researches on the apoptotic and anticancer effects of HP plant extracts and active ingredients (17).

As a similar study, in the study published by Atashi et al. in 2020, it was shown that Hypericin, one of the active ingredients in HP, had an anticancer effect on the Huh7 cell line via the p53 pathway. We aim to find an answer to this gap in the literature, as this study can be confirmed and there is no such study in the HepG2 cell line. (18)

Materials and methods

Cell culture

HepG2 cells (American Type Culture Collection, USA) were cultured in 89% Dulbecco's Modified Eagles' Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotic (Streptomycin+Penicillin). Cultures were performed in an incubator at 37 °C and 5% CO2. Cells were seeded on 11 mm D263M schott glass coverslips placed in the culture dish for morphological analyses, and in 96-well sterile culture dishes with 1x 104 cells per well for cell viability analysis. Cell images were recorded live under a phase contrast microscope every day. For this purpose, live cells were examined under an inverted light microscope (Istanbul Atlas University, Zeiss, Istanbul). 0.8% HP extract was applied to the treatment groups (HP) for 24 hours by completing the medium (19).

Cell viability test

The proliferation measurement of the cells followed in HepG2 cell culture and six different groups was performed with the CCK-8 (WST-8) kit. For this purpose, 1x 104 cells per well were seeded in 96-well sterile culture dishes.

Various metabolic calorimetric methods have been developed to measure proliferation. In addition to advantages such as safety, simplicity, and price, these tests are also preferred in terms of the evaluation of cells grown in microplate wells with the help of an automatic microplate spectrophotometer. Tetrazolium salts are reduced to colored formazan crystals only by metabolically active living cells with the "Succinate tetrazolium reductase" system. Water-soluble tetrazolium (WST-1) in this system, which is active only in living cells, is one of the most well-known tetrazolium salts (20,21).

CCK-8, a cell amount and cytotoxicity detection kit designed on this basis, was used in cell proliferation analysis. The CCK-8 kit uses the water-soluble tetrazolium salt WST-8, which is converted to a water-soluble orange formazan dye by reduction. CCK-8 has low toxicity and enables precise colorimetric measurements for determining the number of viable cells in cell proliferation and cytotoxicity experiments. The amount of formazan dye produced by dehydrogenases in cells is directly proportional to the number of viable cells (22,23). Samples prepared according to the kit procedure were read spectrophotometrically in a microplate reader (BioTek Synergy H1) at the specified wavelength and the resulting color change was evaluated with the obtained optical densities.

Immunohistochemistry

Immunohistochemistry (IHC) was performed to evaluate p53 expression in HepG2 cells. Cells were cultured on sterile coverslips in 24-well plates, fixed during weak adhesion and adhesion periods, and processed according to the IHC staining protocol. After washing with PBS, the cells were incubated in Cello IF (Cellorama) solution for 10 minutes at room temperature. The primary antibody was applied at an appropriate dilution and incubated for 60 minutes at room temperature, followed by washing with Cello IF (Cellorama). The secondary antibody (Goat IgG Mouse DyLight) was then applied and incubated for 60 minutes at room temperature in the dark. After further washing with Cello IF (Cellorama), the cells were mounted using Hoechst nuclear dye and glycerol. Finally, the stained cells were analyzed using a confocal microscope (LSM700 Zeiss).

Statistical analysis

All experiments were repeated 3 times. Data were evaluated using the GraphPad Prism 10.0 analysis system. Unpaired Student's t-test performed, P-value=0.05 was the significant level.

Results

Cell viability and proliferation

Figure 1 shows that a significant decrease in proliferation ($p<0.05$) was detected in HepG2 cells treated with 0.8% extract of HP compared to the control group. According to the CCK8 cell viability analysis, while high levels of cell viability were observed in control group cells, this viability was significantly reduced in cells treated with HP (0.8%). This shows that HP (0.8%) application significantly reduced cell viability by creating a cytotoxic effect in HepG2 cells.

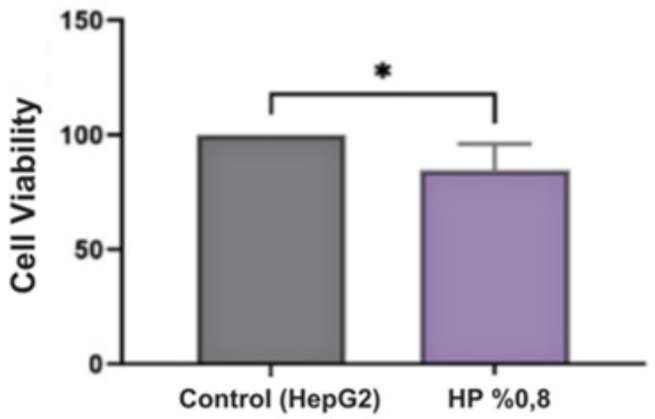

Figure 1: The effect of HP extract (0.8%) on HepG2 cell viability, as assessed by the CCK-8 assay. The bar graph shows a statistically significant decrease in cell viability in the HP-treated group compared to the untreated control group, as indicated by the asterisk.

Immunohistochemical analysis

It is seen from Figure 2 that no significant difference was observed in terms of p53 fluorescence intensity between the control group (HepG2) and the HP (0.8%) group. Although the mean fluorescence intensity of both groups was very close to each other, according to the confocal microscope analysis results, p53 expression in the control group (HepG2) was largely localized in the cytoplasm, while in the HP (0.8%) group, p53 expression was concentrated in the nucleus (Figure 3).

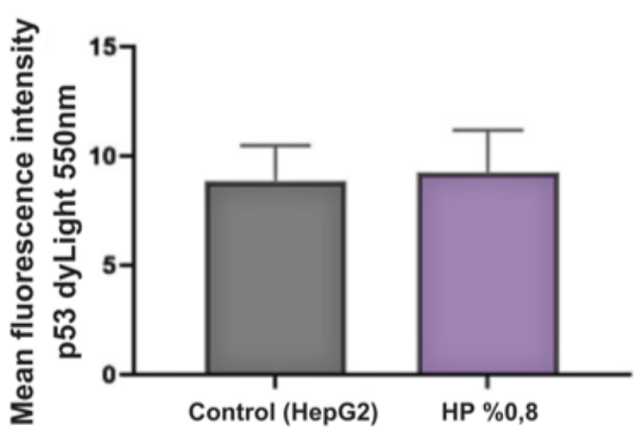

Figure 2: Comparison of p53 immunoreactivity (IR) between the control (HepG2) group and the HP (0.8%) treated group, as measured by mean fluorescence intensity at 550 nm. While both groups exhibit similar fluorescence intensity levels, a slight increase is observed in the HP-treated group, suggesting an enhancement in p53 activation.

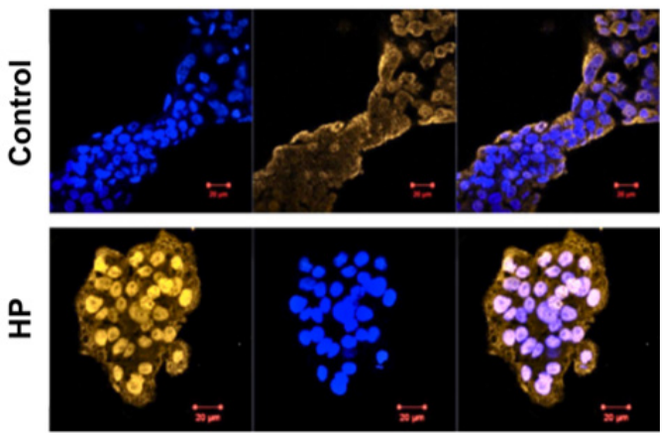

Figure 3: Representative confocal images showing p53 immunofluorescence staining in control and HP-treated HepG2 cells. Nuclei were counterstained with Hoechst. Scale bar: 20 µm.

Discussion

Hepatocellular carcinoma remains a major global health problem due to its high mortality rate, frequent recurrence, and limited therapeutic options (1–4). Therefore, the identification of novel compounds with potential anticancer activity continues to be an important area of research. In this context, natural products and plant-derived extracts have attracted increasing attention because of their diverse bioactive compounds and potential effects on cancer-related cellular pathways (17,20,21).

In the present study, the effect of Hypericum perforatum extract on HepG2 cells was evaluated using a CCK-8 cell viability assay and p53 immunofluorescence staining. The main finding of this study was that treatment with 0.8% Hypericum perforatum extract for 24 hours significantly reduced CCK-8-based cell viability in HepG2 cells compared with the control group. This result suggests that HP extract may impair the metabolic activity and/or proliferation of HepG2 cells. Since the CCK-8 assay reflects the activity of metabolically active viable cells, the observed decrease may indicate a cytostatic or cytotoxic effect of HP extract. However, additional assays are required to distinguish whether this reduction is mainly due to decreased proliferation, increased cell death, or altered metabolic activity.

Hypericum perforatum contains several biologically active secondary metabolites, including hypericin, hyperforin, flavonoids, phenolic compounds, tannins, and other constituents (15,17,22,23). Previous studies have reported that some of these compounds may exert anticancer effects through mechanisms involving oxidative stress, apoptosis, inflammation-related pathways, and modulation of cell survival signaling (17,23). In particular, hypericin has been investigated for its potential effects on cancer cells, including liver cancer models (18,23). Therefore, the reduction in HepG2 cell viability observed in the present study is consistent with previous reports suggesting that HP-derived compounds may affect tumor cell survival (17–19,23).

Another important finding of this study was the apparent change in the subcellular distribution of p53 following HP treatment. Although total p53 fluorescence intensity did not show a marked difference between the control and HP-treated groups, confocal microscopy suggested that p53 immunoreactivity was more concentrated in the nuclear compartment after HP exposure. This observation may be biologically relevant because p53 functions as a transcription factor, and its nuclear localization is associated with the regulation of genes involved in cell cycle arrest, DNA repair, senescence, and apoptosis (13,14,24). Therefore, the nuclear redistribution of p53 observed in HP-treated cells may reflect a cellular stress response.

However, the present findings should be interpreted with caution. Although p53 nuclear localization may suggest increased functional activity, this study did not directly measure p53 transcriptional activity or downstream target genes such as p21, Bax, PUMA, or MDM2. In addition, apoptosis markers, DNA damage markers, and cell cycle analyses were not evaluated. Therefore, it cannot be concluded from the present data alone that HP extract activates a p53-dependent apoptotic pathway. Rather, the findings indicate that HP treatment is associated with reduced cell viability and altered p53 localization in HepG2 cells.

The decrease in cell viability and the redistribution of p53 may be related events, but a causal relationship was not directly demonstrated in this study. To confirm whether p53 plays a functional role in the observed effect of HP extract, future studies should include p53 inhibition, p53 knockdown, or comparison with p53-deficient cell models. Moreover, western blotting or quantitative immunofluorescence analysis could be used to evaluate total p53 expression, phosphorylated p53 levels, and nuclear/cytoplasmic p53 ratios more precisely (13,14).

This study has several limitations. First, only one concentration of HP extract and one treatment duration were evaluated. Therefore, dose-response and time-dependent effects could not be determined. Second, the chemical composition of the HP extract was not characterized in detail. Since HP contains

multiple active compounds, further studies are needed to identify which components are mainly responsible for the observed effect (15,17,22,23). Third, the study was performed using only the HepG2 cell line. Although HepG2 cells are widely used as an in vitro liver cancer model, findings should be confirmed in additional liver cancer cell lines and, eventually, in in vivo models (5–12). Finally, apoptosis, DNA damage, and cell cycle markers were not directly assessed, limiting the mechanistic interpretation of the results.

Overall, the present study suggests that Hypericum perforatum extract may reduce cell viability and alter p53 localization in HepG2 cells. These findings support the need for further investigation of HP extract and its active constituents in liver cancer models. Future studies should include dose-response analyses, chemical characterization of the extract, evaluation of apoptosis and DNA damage markers, and functional experiments targeting the p53 pathway to clarify the underlying molecular mechanisms (13,14,17,18,23).

Limitations

This study has some limitations that should be considered when interpreting the findings. Only a single concentration of Hypericum perforatum extract and one treatment duration were evaluated. In addition, the experiments were performed only in the HepG2 cell line, and apoptosis- or DNA damage-related molecular markers were not analyzed. Therefore, further studies are needed to clarify the underlying mechanisms and confirm these findings in additional models.

Conclusion

In conclusion, Hypericum perforatum extract reduced CCK-8-based cell viability in HepG2 cells after 24 hours of treatment. Immunofluorescence analysis suggested a redistribution of p53 from the cytoplasmic to the nuclear compartment following HP treatment. These findings indicate that HP extract may induce a cellular stress response in HepG2 cells. However, additional dose-response studies and molecular analyses are required to determine whether this effect is mediated by p53-dependent apoptotic pathways.

Conflict of interest

The authors declare that there are no conflicts of interest.

Financial disclosure

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Statement of ethical approval

This study does not require ethics committee approval.

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