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RESEARCH ARTICLE

Effects of Epigallocatechin-3-Gallate in Preventing 5-Fluorouracil-induced Liver Injury in AML-12 Cell Line

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SUMMARY

This study evaluates the potential of epigallocatechin-3-gallate (EGCG) to mitigate 5-fluorouracil (5-FU)-induced hepatotoxicity using the AML-12 cell line. The AML-12 cell line is divided into four groups: control, 5-FU, EGCG, and 5-FU+EGCG. IC50 (Inhibitory Concentration 50) values are determined using the MTT assay. The mRNA expression levels of antioxidant system-related genes, including SOD, CAT, and GSH, are analyzed via RT-qPCR. Additionally, the expression levels of apoptosis-related genes such as Caspase-9 (Cas-9), Apaf-1, Caspase-3 (Cas-3), Bcl-2, and Bax, as well as p53 and SMAC/DIABLO, are evaluated. Co-administration of EGCG with 5-FU results in a significant increase in GSH, SOD, and CAT mRNA expression levels. Treatment with 5-FU alone significantly increases the expression levels of SMAC/DIABLO, Bax, Apaf-1, Bcl-2, and Cas-3 mRNA by inducing apoptosis. Furthermore, co-administration of EGCG and 5-FU leads to a significant elevation in the mRNA expression levels of Cas-9, Bax, Apaf-1, p53, Cas-3, and SMAC/DIABLO, indicating the elimination of damaged structures through apoptosis. In conclusion, our findings demonstrate that EGCG exerts a hepatoprotective effect against 5-FU-induced damage through its antioxidant properties. Moreover, EGCG enhances the anticancer efficacy of 5-FU by promoting apoptosis and facilitating the removal of damaged cells. These results suggest a potential therapeutic synergy between EGCG and 5-FU in treating liver damage and cancer.

Keywords: *5-Fluorouracil, epigallocatechin-3-gallate, hepatoprotective effect, oxidative stress, apoptosis.*

AML-12 Hücre Hattında 5-Florourasil Kaynaklı Karaciğer Hasarının Önlenmesinde Epigallokateşin-3-Gallat'ın Etkileri

ÖZ

Bu çalışmada, AML-12 hücre hattı kullanılarak epigallokateşin-3-gallat'ın (EGCG), 5-florourasil (5-FU) kaynaklı hepatotoksisiteyi azaltma potansiyeli değerlendirilmiştir. AML-12 hücre hattı, kontrol, 5-FU, EGCG ve EGCG+FU olmak üzere dört gruba ayrılmıştır. IC50 (İnhibitör Konsantrasyon 50) değerleri, MTT testi kullanılarak hesaplanmıştır. SOD, katalaz ve GSH gibi antioksidan sistemle ilişkili genlerin mRNA ekspresyon düzeyleri RT-qPCR yöntemiyle analiz edilmiştir. Ayrıca, apoptozla ilişkili Kaspaz-9 (Cas-9), Apaf-1, Kaspaz-3 (Cas-3), Bcl-2 ve Bax genlerinin ekspresyon düzeyleri ile birlikte p53 ve SMAC/DIABLO gen ekspresyonları da değerlendirilmiştir. EGCG'nin, 5-FU ile birlikte uygulanması sonucunda, GSH, SOD ve CAT mRNA ekspresyon düzeylerinde anlamlı bir artış tespit edilmiştir. 5-FU uygulamasının, apoptozu uyararak SMAC/DIABLO, Bax, Apaf-1, Bcl-2 ve Cas-3 mRNA ekspresyon düzeylerini istatistiksel olarak anlamlı şekilde artırdığı gözlenmiştir. EGCG'nin, 5-FU ile birlikte uygulanması ise Cas-9, Bax, Apaf-1, p53, Cas-3 ve SMAC/DIABLO mRNA ekspresyon düzeylerinde anlamlı artışa neden olmuş, bu durumun hasarlı yapıların apoptoz yoluyla ortadan kaldırıldığını gösterdiği anlaşılmıştır. Sonuç olarak, bulgularımız EGCG'nin antioksidan özellikleri sayesinde 5-FU kaynaklı hasara karşı hepatoprotektif etkiler sağladığını göstermektedir. Ayrıca, EGCG'nin apoptozu teşvik ederek hasarlı hücrelerin ortadan kaldırılmasını kolaylaştırdığı ve 5-FU'nun antikanser etkinliğini artırdığı ortaya konmuştur. Bu sonuçlar, karaciğer hasarı ve kanser tedavisinde EGCG ve 5-FU arasında potansiyel bir terapötik sinerji bulunduğunu düşündürmektedir.

Anahtar Kelimeler: *5-Florourasil, epigallokateşin-3-gallat, hepatoprotektif etki, oksidatif stres, apoptoz.*

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INTRODUCTION

Cancer comprises a collection of prevalent diseases marked by unregulated cellular proliferation. Surgery, radiation, and chemotherapy are widely utilized as cancer treatment options (Cleeland et al., 2012; Herrmann et al., 2020; Dongsar et al., 2023). Chemotherapy is essential in cancer treatment, targeting the eradication or suppression of cancer cell proliferation. However, chemotherapy resistance remains a significant obstacle, impairing the effectiveness of treatment and reducing its therapeutic potential. Contemporary cancer therapies, aside from their exorbitant expense, engage with signaling pathways, leading to a diverse array of side effects with differing severities and classifications. These side effects depend on the dosage of chemotherapeutic drugs and the patient's sensitivity to the medications. Due to their non-selective nature, chemotherapeutic drugs damage healthy tissues and cells alongside tumor cells (Wang et al., 2023).

Cytotoxic drugs, which represent an important class of chemotherapy, demonstrate high efficacy in cancer treatment. 5-fluorouracil (5-FU) is a prominent cytotoxic drug (Ranjit et al., 2023). It permanently inhibits thymidylate synthase (Yu et al., 2015). 5-FU-based therapies are widely utilized as a key component in various chemotherapy regimens for cancer treatment. However, 5-FU is associated with hepatoxicity, including hepatitis, steatohepatitis, and hepatic sinusoidal obstruction syndrome (Vauthey et al., 2006; Robinson et al., 2012; Hubert et al., 2013). Hepatocellular damage is reported to intensify due to elevated aminotransferases in 5-FU-induced liver cirrhosis (Momiyam et al., 2015).

Certain phytochemicals, such as epigallocatechin gallate (EGCG), enhance sensitivity to chemotherapy and mitigate chemotherapy-induced toxic side effects (Wang et al., 2023). EGCG exhibits various health advantages (Alam et al., 2022). Depending on its dosage, EGCG is reported to exhibit either antioxidant or pro-oxidant properties (Yang et al., 2022). It also exhibits antioxidant, anti-inflammatory, anti-allergic, anti-angiogenesis, vasodilator, and anti-carcinogenic effects (Liczbiński et al., 2022).

This study investigates the impact of EGCG on 5-FU-induced hepatotoxicity. The study investigates the mRNA levels of key antioxidant enzymes, such as glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). Furthermore, it explores the gene expression profiles related to apoptosis, including SMAC/DIABLO (Second Mitochondria-Derived Activator of Caspases/Direct IAP Binding Protein with Low pI), Bcl-2 (B-cell lymphoma 2), Caspase-9 (Cas-9), Apaf-1 (Apoptotic Protease-Activating Factor 1), p53 (tumor suppressor protein 53), Caspase-3 (Cas-3), and Bax (Bcl-2-associated X protein). The effects of pathways involved in the apoptotic process are analyzed to elucidate their influence on gene expression.

MATERIALS AND METHODS

Groups

This study includes four experimental groups: control, 5-FU, EGCG, and 5-FU+EGCG.

Cell culture

AML-12 cells (ATCC®, CRL-2254 TM) are cultured in flasks containing a nutrient medium composed of 10 mg/ml streptomycin, 100 IU/ml penicillin, 1% L-glutamine, and 5% heat-inactivated fetal bovine serum. The medium is prepared in a 1:1 ratio of Dulbecco's Modified Eagle's Medium, HAMS F12, and Eagle's Minimum Essential Medium. The study uses cells between the 5th and 12th passages (5% CO_2, 37°C).

Determination of EGCG and 5-FU Dosages

The dosages of EGCG and 5-FU are determined based on prior studies. In vitro research indicates a wide range of IC50 (Inhibitory Concentration 50) values for 5-FU, varying with cell line and experimental conditions. For example, the IC50 of 5-FU is approximately 13 μg/ml in SW620 colon cancer cells (Gao et al., 2014) and ranges from 0.25

μM to 1.5 μM in MCF7 breast cancer cells when used with β-escin (Mazrouei et al., 2019). Similarly, EGCG at a concentration of 20 μM has been shown to augment the apoptotic effects of 5-FU in MCF7 cells (Zhang et al., 2016). Based on these findings and the requirements of the experimental design, 5-FU and EGCG are administered at concentrations between 1.25 and 20 μM in this study, enabling a comprehensive evaluation of their effects on the AML-12 cell line.

Determination of IC50 doses by the MTT assay

Percent viability is calculated using the Thiazolyl Blue Tetrazolium Bromide (MTT) assay, and IC50 values are determined via probit analysis (Turker and Bakar, 2023). A total of 180 μL of $1x10^6$ cells are inoculated into each well of 96-well plates, with four replicates studied. At the end of the 24 hours of incubation (37°C, 5% CO_2) 20 μL of the agents indicated for each group are applied and incubated for an additional 24 hours. An aqueous solution containing 0.01% dimethyl sulfoxide (DMSO) was added to the control group. Aqueous solutions of 5-FU (Sigma F6627) and EGCG (Sigma 1236700), each containing 0.01% DMSO, are prepared separately. 5-FU and EGCG are combined in a 1:1 (v/v) ratio and administered simultaneously to the treatment groups. All substances, except in the control group, are applied at varying doses of 1.25, 2.5, 5, 10, and 20 μM, with a total volume of 20 μL. The cells in all groups are exposed to the substances for a total duration of 24 hours. Thereafter, 20 μL of MTT solution (5 mg/mL) is introduced into the wells, and the plates are left to incubate (3 hours). 200 μL of 0.01% DMSO is added to dissolve the resultant formazan crystals. Absorbance values are recorded at 492 nm using a microplate reader. The viability of cells in the control group is assumed to be 100%, and the IC50 doses are determined using probit analysis.

RNA isolation and cDNA synthesis

The AML-12 cell line is plated in culture dishes with $3x10^6$ cells allocated to each well and maintained under incubation for 24 hours. Subsequently, the chemical compounds are applied to the cells at their determined IC50 values and left to incubate (24 hours). Following treatment, RNA is extracted from the cells using the PureLink RNA Mini Kit. The quality and amount of RNA obtained are evaluated using a Nanodrop spectrophotometer. cDNA synthesis is subsequently carried out using the High Capacity cDNA Reverse Transcription Kit.

RT-qPCR analysis

Real-time Quantitative polymerase chain reaction (RT-qPCR) analysis is conducted using the method described by Akıncı et al. (Akıncı et al., 2023). Primer sequences are provided in Table 1. This analysis assesses the expression levels of antioxidant enzymes, such as SOD, CAT, and GSH, as well as genes associated with apoptosis, such as Apaf-1, SMAC/DIABLO, Bax, Cas-9, Bcl-2, p53, and Cas-3. mRNA expression is evaluated through the comparative cycle threshold method (2^−ΔΔCt) (Akıncı et al., 2023). Gene expression levels are analyzed relative to the control group and standardized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA as a reference.

Table 1. Primer sequences of the genes used for RT-qPCR analysis

Gene	Primer sequences (Forward/Reverse)
SOD	5'-AGCTGCACCACAGCAAGCAC-3' (Tam et al. 2023) 5'-TCCACCACCCTTAGGGCTCA-3'
CAT	5'-TCCGGGATCTTTTTAACGCCATTG-3' (Dkhil et al. 2016) 5'-TCGAGCACGGTAGGGACAGTTCAC-3'
GSH	5'-ACTTGGCACTCCTCTCCTGA-3' (Akıncı et al. 2023) 5'-AGGCACTAGAACCTGCTGGA-3'
Cas-3	5'-GGTATTGAGACAGACAGTGG-3' (Oltulu et al. 2022) 5'-CATGGGATCTGTTTCTTTGC-3'
Cas-9	5'-GAGTCAGGCTCTTCCTTTG-3' (Oltulu et al. 2022) 5'-CCTCAAACTCTCAAGAGCAC-3'
Apaf-1	5'-GATATGGAATGTCTCAGATGGCC-3' (Yakovlev et al. 2001) 5'-GGTCTGTGAGGACTCCCCA-3'
Bax	5'-TTCATCCAGGATCGAGCAGA-3' (Oltulu et al. 2022) 5'-GCAAAGTAGAAGGCAACG-3'
Bcl-2	5'-ATGTGTGTGGAGAGCGTCAA-3' (Oltulu et al. 2022) 5'-ACAGTTCCACAAAGGCATCC-3'
p53	5'-CACGAGCGCTGCTCAGATAGC-3' (Oltulu et al. 2022) 5'-ACAGGCACAAACACGCACAAA-3'
SMAC/DIABLO	5'-CTCTGTGGCTGAGGGTTGAT-3' (Tokatlı et al. 2020) 5'-TTGTAGATGATGCCCACAGG -3'
GAPDH	5'-GTCTCCTCTGACTTCAACAGCG-3' (Bednarz-Misa et al. 2020) 5'-ACCACCCTGTTGCTGTAGCCAA-3'

Statistical analysis

IC50 values were determined by probit analysis using MTT assay data. The AML-12 cell line is subsequently treated with the IC50 doses of EGCG and 5-FU, which are calculated as 0.38 μM for EGCG and, 4.78 μM for 5-FU respectively, for 24 hours. The relative fold-change values of gene expressions are analyzed using one-way *ANOVA* followed by post hoc *Tukey's test*, with statistical significance set at $p < 0.05$. Probit analysis and *ANOVA* tests are performed using SPSS 20 software (IBM).

RESULTS

MTT Assay

The MTT assay evaluates the effects of 5-FU, EGCG, and their combination (5-FU+EGCG) on the viability of AML-12 cells. The cells are incubated for 24 hours before the MTT assay. The results indicate a dose-dependent decline in cell viability across all treatment groups (5-FU, EGCG, and 5-FU+EGCG) compared to the control group (Figure 1). The IC50 values are presented in Figure 1.

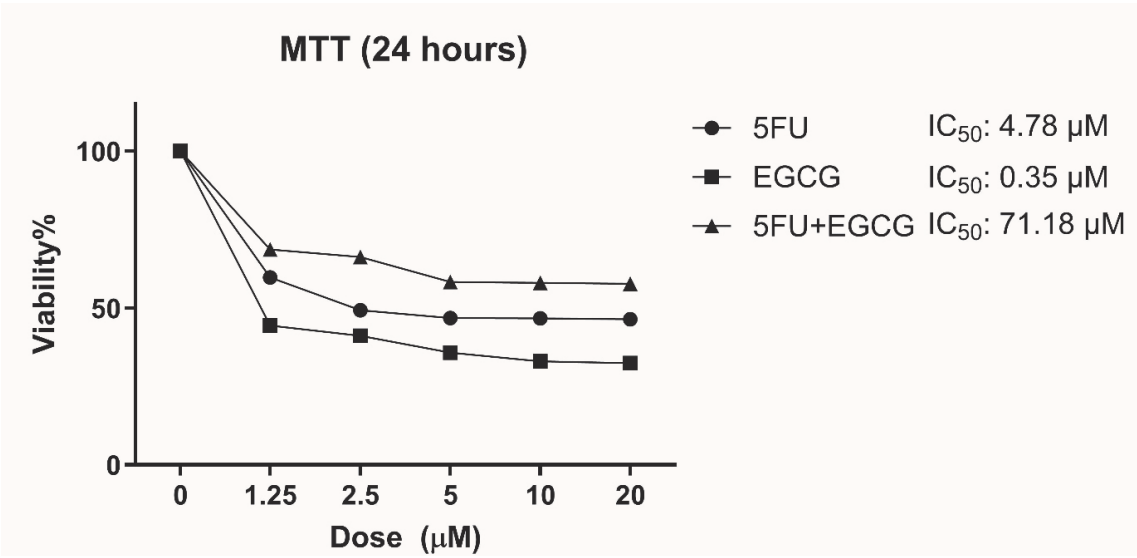

Figure 1. MTT assay results. The standard deviation is shown by vertical bars. (mean ±std dev.) (The percentage of viability is calculated using the formula: (average absorbance of the sample/average absorbance of the control) × 100.).

Antioxidant Gene mRNA Expression

CAT, SOD, and GSH are analyzed. SOD mRNA expression significantly increases in all treatment groups (EGCG, 5-FU, and 5-FU+EGCG) against the control group, with the combination group (5-FU+EGCG) exhibiting the highest expression levels (Figure 2A). Similar trends are observed for CAT and GSH mRNA expression levels, where the combination group reveals a considerable improvement over the control and single-treatment groups (Figures 2B and 2C). These findings indicate that the combined application of EGCG and 5-FU enhances the antioxidant defense mechanism.

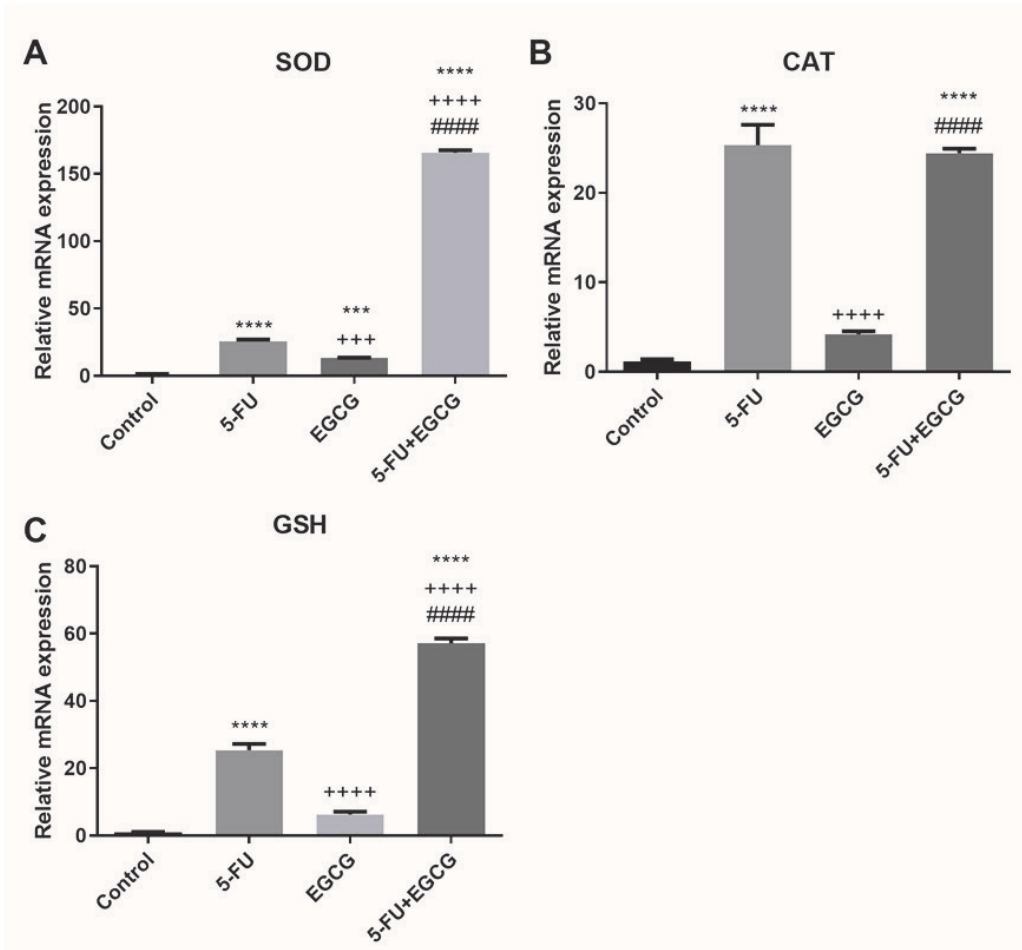

Figure 2. SOD (A), CAT (B), GSH (C) Relative mRNA Expression. **** p<0.0001, *** p<0.001 compared to control group, ++++ p<0.0001, +++ p<0.001 compared to 5-FU group, #### p<0.0001, ### p<0.001 compared to EGCG group.

Apoptosis-Related Gene mRNA Expression

The expression levels of apoptosis-related genes Bax, Apoptotic protease-activating factor 1 (Apaf-1), Bcl-2, Cas-9, p53, Cas-3, and SMAC/DIABLO are evaluated. Treatment with 5-FU significantly increases the expression of pro-apoptotic genes (Cas-3, SMAC/DIABLO, Cas-9, Bax, and Apaf-1), while anti-apoptotic Bcl-2 expression decreases against the control group (Figure 3). Co-administration of EGCG and 5-FU leads to further upregulation of pro-apoptotic genes and p53 expression, suggesting enhanced apoptosis in the combination group (Figures 3A-3G). These findings imply that EGCG amplifies the apoptotic effects of 5-FU by regulating both anti- and pro-apoptotic pathways.

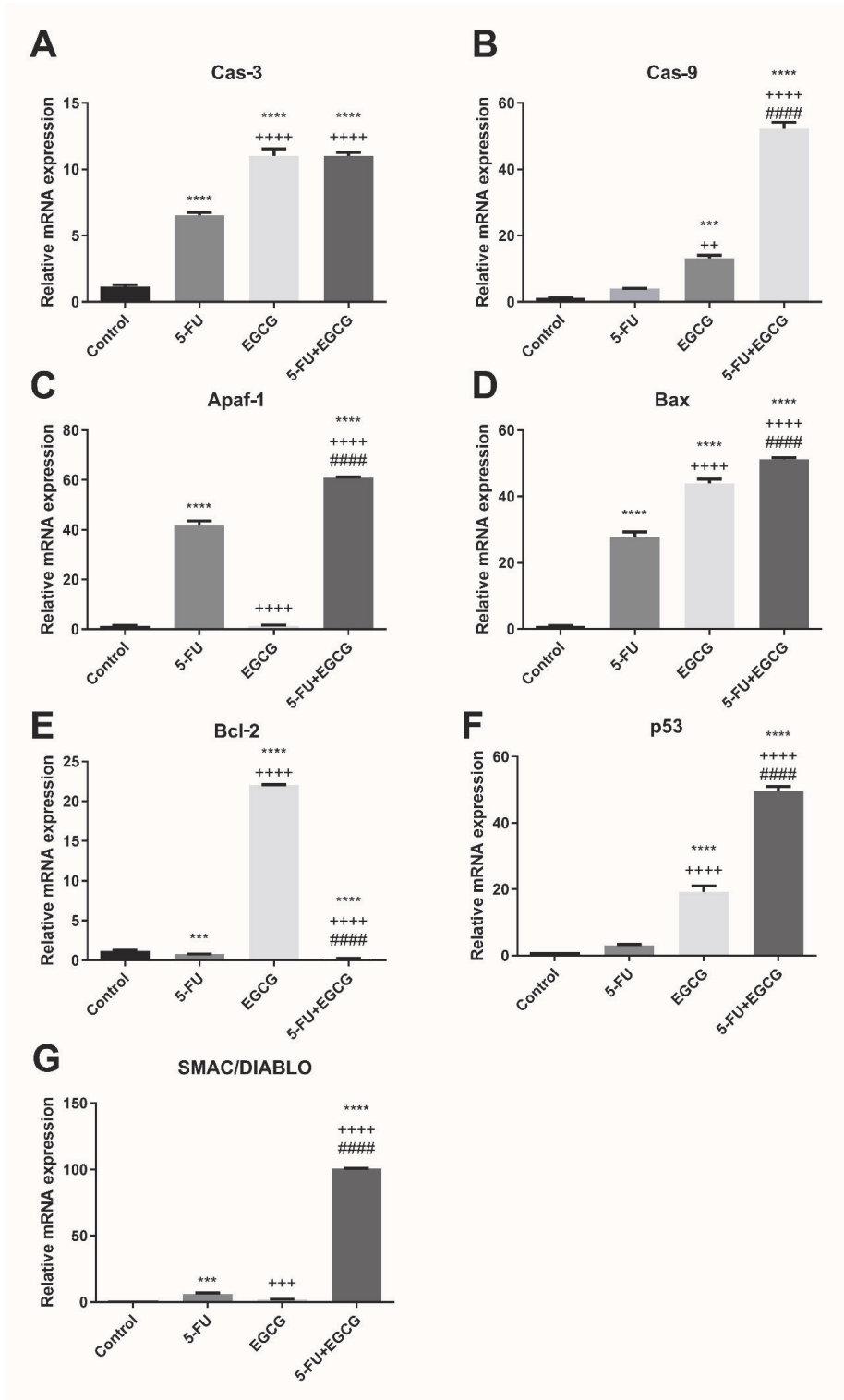

Figure 3. Cas-3 (A), Cas-9 (B), Apaf-1 (C), Bax (D), Bcl-2 (E), p53 (F), SMAC/DIABLO (G) Relative mRNA Expression. **** p<0.0001, *** p<0.001 comparing to control group, ++++ p<0.0001, +++ p<0.001 comparing to 5-FU group, #### p<0.0001 comparing to EGCG group.

DISCUSSION

The liver constitutes a vital organ responsible for maintaining health and homeostasis. It performs critical roles in numerous biochemical processes, including growth, disease defense, nutrient provisioning, and energy production. The primary functions of the liver include carbohydrate, protein, and fat metabolism, bile secretion, vitamin storage, and detoxification. A healthy liver is therefore indispensable for overall well-being. Hepatotoxicity denotes liver injury induced by various chemicals. Certain medications, particularly at high doses or in predisposed individuals, induce hepatotoxicity. Examples of hepatotoxins include acetaminophen and alcohol, along with herbal products and industrial chemicals. These agents damage hepatocytes, leading to dysfunction and potentially causing hepatitis, jaundice, liver fibrosis, or alcoholic liver disease. The liver injury induced by the drug is estimated to represent around 5% of all hospital admissions for acute liver failure (Pandit et al., 2012).

5-FU is commonly used in the treatment of various cancers. It is used either as monotherapy or in conjunction with other medications (Pujari and Bandawane, 2021). 5-FU, classified as an antimetabolic drug, influences the synthesis of RNA and DNA in both tumor and normal cells. The predominant portion of 5-FU undergoes detoxification via the liver, with only a minor quantity excreted through the kidneys (Gelen et al., 2018). Toxic intermediates responsible for liver injury are produced during 5-FU metabolism. Studies demonstrate that 5-FU treatment induces oxidative stress in the liver, resulting in structural and functional abnormalities in hepatocytes, as observed in both in vitro and in vivo investigations (Tam et al., 2003). In response to 5-FU, increased activity levels of ALP (Alkaline Phosphatase), lactate dehydrogenase, and AST (Aspartate Aminotransferase) are observed (Gelen et al., 2018).

Traditional medicines have historically been used to treat liver ailments. The hepatoprotective benefits of these remedies are often ascribed to their antioxidative characteristics and their ability to stimulate the body's innate antioxidative defense system. Given the role of oxidative stress in nearly all forms of liver damage, the antioxidative properties of these substances likely play a significant role in their hepatoprotective effects. Increasing data indicates that the therapeutic activity of natural substances is attributable to pharmacological qualities beyond antioxidative mechanisms (Domitrović and Potočnjak, 2016).

EGCG, the primary flavonoid in tea, is shown in numerous studies to mitigate drug-induced liver injury, although its precise mechanism of action remains unclear. For instance, in an experimental study conducted by Lin et al. in rats, EGCG demonstrates antioxidant activity that reduces acetaminophen-induced liver damage (Lin et al., 2021). This study focuses on EGCG's ability to prevent 5-FU-induced liver damage.

The increase in antioxidant enzyme mRNA expression levels (SOD, CAT, and GSH) observed in our study aligns with findings from Gelen et al. (Gelen et al., 2018), which demonstrate the depletion of these enzymes under 5-FU-induced oxidative stress. By enhancing their expression, EGCG appears to counteract the oxidative damage induced by 5-FU, consistent with the hepatoprotective effects reported by Lin et al. in acetaminophen-induced liver injury (Lin et al., 2021).

Recent findings reveal that 5-FU-induced liver and kidney damage is associated with elevated ROS levels. Antioxidant levels, such as SOD, CAT, and GSH, are shown to decrease in liver and kidney tissues following 5-FU administration. Furthermore, serum malondialdehyde (MDA) levels increase significantly. Studies in experimental animals indicate that 5-FU administration lowers SOD and GSH levels, accelerates lipid peroxidation, and significantly elevates serum ALT (Alanine Aminotransferase), AST, and ALP activity, leading to hepatotoxicity (Gelen et al., 2018).

EGCG, known for its anti-inflammatory and antioxidant activities, exhibits anticancer properties in animal studies. EGCG inhibits the proliferation of hepatocellular carcinoma cells and promotes apoptosis (Yang et al. 2012). Yang et al. (2012) demonstrate that EGCG enhances the suppression of cell proliferation induced by 5-FU in hepatocellular carcinoma cells. EGCG enhances the susceptibility of hepatocellular carcinoma cells to the anticancer properties of 5-FU. Moreover, EGCG and 5-FU exhibit a synergistic effect on chemoresistant cancer cells (Moracci et al., 2022). This study demonstrates that co-administration of EGCG with 5-FU significantly increases the mRNA expression levels of SOD, CAT, and GSH, supporting the antioxidant defense system.

Apoptosis, a physiological process that selectively eliminates undesirable cells, plays a pathological role in cases of cell injury. Cytotoxic or chemotherapeutic drug exposure induces oxidative stress, causing cell damage. Cancer progression can be mitigated by eliminating damaged cells through apoptosis (Coşkun and Özgür, 2011). DNA damage activates p53, a transcription factor that allows time for DNA repair while suppressing anti-apoptotic factors and promoting pro-apoptotic factors (Gökhan et al., 2020). Anti-apoptotic effectors like Bcl-2 are downregulated, while pro-apoptotic effectors such as Bax are released. Bax/Bak proteins oligomerize and translocate to the mitochondria, promoting outer membrane permeabilization. This facilitates the cytoplasmic translocation of pro-apoptotic proteins such as cytochrome c and SMAC/DIABLO. Cytochrome c and Apaf-1 form the apoptosome, which activates Cas-9 and subsequently Cas-3 (Pradhan et al., 2023). The upregulation of pro-apoptotic genes (SMAC/DIABLO, Bax, Apaf-1, Cas-3, and Cas-9) and the downregulation of anti-apoptotic Bcl-2, observed upon 5-FU treatment, are consistent with apoptosis induction mechanisms described by Coşkun and Özgür (Coşkun and Özgür, 2011) and Pradhan et al. (Pradhan et al., 2023). The enhanced apoptotic response when EGCG is co-administered highlights its role in amplifying chemotherapeutic efficacy, as similarly demonstrated by Moracci et al. (Moracci et al., 2022) in chemoresistant cancer cells.

Our study builds upon the existing literature by providing comprehensive data on the dual role of EGCG in mitigating 5-FU-induced hepatotoxicity while enhancing its anticancer activity. Previous research has demonstrated the antioxidant properties of EGCG in preventing oxidative stress-induced liver damage (Lin et al., 2021) and its ability to enhance the cytotoxic effects of 5-FU in cancer models (Yang et al., 2012; Moracci et al., 2022). However, the precise mechanisms underlying these effects, particularly in the context of apoptosis and antioxidant gene modulation, remain poorly understood.

Our findings provide novel insights into the molecular pathways involved, demonstrating significant upregulation of key antioxidant enzymes (SOD, CAT, and GSH) and pro-apoptotic genes (SMAC/DIABLO, Bax, Apaf-1, Cas-9, and Cas-3) when EGCG is co-administered with 5-FU. This suggests that EGCG not only mitigates oxidative damage but also promotes the removal of damaged cells through apoptosis, thereby reducing the hepatotoxic effects of 5-FU and potentially enhancing its therapeutic efficacy.

By targeting both oxidative stress and apoptotic pathways, EGCG demonstrates a dual protective and synergistic effect when combined with 5-FU. These findings advance our understanding of the therapeutic synergy between natural compounds like EGCG and conventional chemotherapeutics such as 5-FU. They also validate the role of natural compounds in mitigating drug-induced toxicities, while emphasizing their potential to enhance cancer treatment outcomes. This study thus provides a foundation for future in vivo investigations and clinical trials to evaluate EGCG as a promising adjuvant therapy in cancer treatment.

CONCLUSION

The findings of this study demonstrate that EGCG protects the liver from 5-FU-induced damage through its antioxidant effects. Additionally, EGCG enhances the anticancer efficacy of 5-FU by inducing apoptosis, thereby facilitating the elimination of cells damaged by 5-FU. These results suggest that further in vivo studies and clinical research are warranted to evaluate the potential of EGCG in mitigating hepatotoxicity and enhancing cancer treatment outcomes.

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AUTHOR CONTRIBUTION STATEMENT

Concept, Design, Supervision, Resources, Materials, Data Collection and Processing, Analysis and Interpretation, Literature Search, Writing, Reviews (MA), Concept, Design, Resources, Materials, Data Collection and Processing, Analysis and Interpretation, Literature Search, Critical Reviews (ÇO), Concept, Design, Resources, Materials, Data Collection and Processing, Analysis and Interpretation, Literature Search, Critical Reviews (EB), Analysis and/or Interpretation, Literature Search, Writing, Critical Reviews (ZAÇY).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Akıncı, M., Oltulu, C., Bakar, E., & Cevikelli Yakut, Z. A. (2023). Puerarin protects from methotrexate induced hepatotoxicity in AML-12 cells. *Nam Kem Medical Journal, 11*(3), 193–201. https://doi.org/10.4274/nkmj.galenos.2023.27147

Alam, M., Ali, S., Ashraf, G. M., Bilgrami, A. L., Yadav, D. K., & Hassan, M. I. (2022). Epigallocatechin 3-gallate: From green tea to cancer therapeutics. *Food Chemistry, 379*, Article 132135. https://doi.org/10.1016/j.foodchem.2022.132135

Bednarz-Misa, I., Neubauer, K., Zacharska, E., Kapturkiewicz, B., & Krzystek-Korpacka, M. (2020). Whole blood ACTB, B2M and GAPDH expression reflects activity of inflammatory bowel disease, advancement of colorectal cancer, and correlates with circulating inflammatory and angiogenic factors: Relevance for real-time quantitative PCR. *Advances in Clinical and Experimental Medicine, 29*(5), 547–556. https://doi.org/10.17219/acem/118845

Cleeland, C. S., Allen, J. D., Roberts, S. A., Brell, J. M., Giralt, S. A., Khakoo, A. Y., & Skillings, J. (2012). Reducing the toxicity of cancer therapy: Recognizing needs, taking action. *Nature Reviews Clinical Oncology, 9*(8), 471–478. https://doi.org/10.1038/nrclinonc.2012.99

Coşkun, G., & Özgür, H. (2011). Apoptoz ve nekrozun moleküler mekanizması. *Arşiv, 20*, 145.

Dkhil, M. A., Moneim, A. E. A., & Al-Quraishy, S. A. (2016). Indigofera oblongifolia ameliorates lead acetate-induced testicular oxidative damage and apoptosis in a rat model. *Biological Trace Element Research, 173*, 354–361. https://doi.org/10.1007/s12011-016-0689-0

Domitrović, R., & Potočnjak, I. (2016). A comprehensive overview of hepatoprotective natural compounds: Mechanism of action and clinical perspectives. *Archives of Toxicology, 90*, 39–79. https://doi.org/10.1007/s00204-015-1580-z

Dongsar, T. T., Dongsar, T. S., Gupta, N., Almalki, W. H., Sahebkar, A., & Kesharwani, P. (2023). Emerging potential of 5-fluorouracil-loaded chitosan nanoparticles in cancer therapy. *Journal of Drug Delivery Science and Technology*, Article 104371. https://doi.org/10.1016/j.jddst.2023.104371

Gao, L., Shen, L., Yu, M., Ni, J., Xiao, D., Zhou, Y., & Wu, S. (2014). Colon cancer cells treated with 5-fluorouracil exhibit changes in polylactosamine-type n-glycans. *Molecular Medicine Reports, 9*(5), 1697–1702. https://doi.org/10.3892/mmr.2014.2008

Gelen, V., Şengül, E., Yıldırım, S., & Atila, G. (2018). The protective effects of naringin against 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats. *Iranian Journal of Basic Medical Sciences, 21*(4), 404–410. https://doi.org/10.22038/IJBMS.2018.27510.6714

Gökhan, A., Kılıç, K. D., Gülle, K., Uyanıkgil, Y., & Çavuşoğlu, T. (2020). Apoptotic pathways and targeted therapies. *Medical Journal of SDU, 27*(4), 565–573. https://doi.org/10.17343/sdutfd.619417

Herrmann, J. (2020). Vascular toxic effects of cancer therapies. *Nature Reviews Cardiology, 17*(8), 503–522. https://doi.org/10.1038/s41569-020-0347-2

Hubert, C., Sempoux, C., Humblet, Y., van den Eynde, M., Zech, F., Leclercq, I., & Gigot, J. F. (2013). Sinusoidal obstruction syndrome (SOS) related to chemotherapy for colorectal liver metastases: Factors predictive of severe SOS lesions and protective effect of bevacizumab. *HPB, 15*(11), 858–864. https://doi.org/10.1111/hpb.12047

Liczbiński, P., & Bukowska, B. (2022). Tea and coffee polyphenols and their biological properties based on the latest in vitro investigations. *Industrial Crops and Products, 175*, Article 114265. https://doi.org/10.1016/j.indcrop.2021.114265

Lin, Y., Huang, J., Gao, T., Wu, Y., Huang, D., Yan, F., & Weng, Z. (2021). Preliminary study on hepatoprotective effect and mechanism of (-)-epigallocatechin-3-gallate against acetaminophen-induced liver injury in rats. *Iranian Journal of Pharmaceutical Research, 20*(3), 46–56. https://doi.org/10.22037/ijpr.2020.112727.13918

Mazrouei, R., Raeisi, E., Lemoigne, Y., & Heidarian, E. (2019). Activation of p53 gene expression and synergistic antiproliferative effects of 5-fluorouracil and β-escin on MCF7 cells. *Journal of Medical Signals & Sensors, 9*(3), 196–202. https://doi.org/10.4103/jmss.jmss_44_18

Momiyam, K., Nagai, H., Ogino, Y., Mukozu, T., Matsui, D., Matsui, T., & ... (2015). Glutathione for hepatotoxicity in patients with liver cirrhosis and advanced hepatocellular carcinoma receiving hepatic arterial infusion chemotherapy. *Clinical Cancer Drugs, 2*(1), 54–60. https://doi.org/10.1007/s00280-014-2564-z

Moracci, L., Sensi, F., Biccari, A., Crotti, S., Gaio, E., Benetti, F., Traldi, P., Pucciarelli, S., & Agostini, M. (2022). An investigation on [5 fluorouracil and epigallocatechin-3-gallate] complex activity on HT-29 cell death and its stability in gastrointestinal fluid. *Oncotarget, 13*, 476–489. https://doi.org/10.18632/oncotarget.28207

Oltulu, C., Akıncı, M., & Elvan, B. (2022). Antitumor activity of etoposide, puerarin, galangin and their combinations in neuroblastoma cells. *International Journal of Life Sciences and Biotechnology, 5*(3), 407–423. https://doi.org/10.38001/ijlsb.1089164

Pandit, A., Sachdeva, T., & Bafna, P. (2012). Drug-induced hepatotoxicity: A review. *Journal of Applied Pharmaceutical Science, 2*(5), 233–243. https://doi.org/10.7324/JAPS.2012.2541

Pradhan, A., Sengupta, S., Sengupta, R., & Chatterjee, M. (2023). Attenuation of methotrexate induced hepatotoxicity by epigallocatechin 3-gallate. *Drug and Chemical Toxicology, 46*(4), 717–725. https://doi.org/10.1080/01480545.2022.2085738

Pujari, R., & Bandawane, D. D. (2021). Hepatoprotective activity of gentisic acid on 5-fluorouracil-induced hepatotoxicity in Wistar rats. *Turkish Journal of Pharmaceutical Sciences, 18*(3), 332–338. https://doi.org/10.4274/tjps.galenos.2020.95870

Ranjit, S., Anjleena, M., & Ranju, B. (2023). Synthetic cytotoxic drugs as cancer chemotherapeutic agents. In P. C. Acharya & M. Kurosu (Eds.), *Medicinal chemistry of chemotherapeutic agents* (pp. 499–537). Academic Press. https://doi.org/10.1016/B978-0-323-90575-6.00015-5

Robinson, S. M., Wilson, C. H., Burt, A. D., Manas, D. M., & White, S. A. (2012). Chemotherapy-associated liver injury in patients with colorectal liver metastases: A systematic review and meta-analysis. *Annals of Surgical Oncology, 19*, 4287–4299. https://doi.org/10.1245/s10434-012-2438-8

Tam, N. N. C., Gao, Y., Leung, Y. K., & Ho, S. M. (2003). Androgenic regulation of oxidative stress in the rat prostate involvement of NAD(P)H oxidases and antioxidant defense machinery during prostatic involution and regrowth. *American Journal of Pathology, 163*(6), 2513–2522. https://doi.org/10.1016/S0002-9440(10)63606-1

Tokatlı, C., Doğanlar, O., & Doğalar, Z. B. (2020). Meriç Delta balıklarında çevre kirliliğinin genotoksik etkileri: Antioksidan savunma, ısı şok protein sinyali ve DNA hasar-onarım mekanizmaları. *Journal of Limnology and Freshwater Fisheries Research, 6*(1), 14–24.

Turker, N. P., & Bakar, E. (2023). Effects of L-dopa and p-coumaric acid combination on oxidative stress, DNA damage, and mitochondrial apoptosis in neuroblastoma cells. *Bangladesh Journal of Pharmacology, 18*(2), 49–57. https://doi.org/10.3329/bjp.v18i2.65531

Vauthey, J. N., Pawlik, T. M., Ribero, D., & ... (2006). Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *Journal of Clinical Oncology, 24*(13), 2065–2072. https://doi.org/10.1200/JCO.2005.05.3074

Wang, L., Li, P., & Feng, K. (2023). EGCG adjuvant chemotherapy: Current status and future perspectives of radiopharmaceuticals in China. *European Journal of Medicinal Chemistry, 250*, Article 115197. https://doi.org/10.1007/s00259-021-05615-6

Yakovlev, A. G., Ota, K., Wang, G., Movsesyan, V., Bao, W. L., & Yoshihara, K. (2001). Differential expression of apoptotic protease-activating factor-1 and caspase-3 genes and susceptibility to apoptosis during brain development and after traumatic brain injury. *The Journal of Neuroscience, 21*(19), 7439–7446. https://doi.org/10.1523/JNEUROSCI.21-19-07439.2001

Yang, L., Jia, L., Li, X., Zhang, K., Wang, X., He, Y., & ... (2022). Prooxidant activity-based guideline for a beneficial combination of (−)-epigallocatechin-3-gallate and chlorogenic acid. *Food Chemistry, 386*, Article 132812. https://doi.org/10.1016/j.foodchem.2022.132812

Yang, X. W., Wang, X. L., Cao, L. Q., Jiang, X. F., Peng, H. P., Lin, S. M., Xue, P., & Chen, D. (2012). Green tea polyphenol epigallocatechin-3-gallate enhances 5-fluorouracil-induced cell growth inhibition of hepatocellular carcinoma cells. *Hepatology Research, 42*(5), 494–501. https://doi.org/10.1111/j.1872-034X.2011.00947.x

Yu, H., Liu, Y., Pan, W., & Shen, S. (2015). Polyunsaturated fatty acids augment tumoricidal action of 5-fluorouracil on gastric cancer cells by their action on vascular endothelial growth factor, tumor necrosis factor-alpha and lipid metabolism related factors. *Archives of Medical Science, 11*(2), 282–291. https://doi.org/10.5114/aoms.2015.50962

Zhang, X., Wang, J., Hu, J., Huang, Y., Wu, X., Zi, C., & Sheng, J. (2016). Synthesis and biological testing of novel glucosylated epigallocatechin gallate (EGCG) derivatives. *Molecules, 21*(5), Article 620. https://doi.org/10.3390/molecules21050620