Molecular Pharmacology of Epigallocatechin Gallate in Oxidative Stress–Mediated Cellular Damage: Antioxidant, Mitochondrial, and Anti-Apoptotic Actions

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ABSTRACT

Background: Oxidative stress plays a pivotal role in the pathogenesis of numerous chronic and degenerative diseases by inducing cellular injury, mitochondrial dysfunction, and apoptosis. Epigallocatechin gallate, a major polyphenolic constituent of green tea, has been widely recognised for its antioxidant potential, yet its integrated cytoprotective mechanisms require further clarification.

Methods: The present study evaluated the cytoprotective effects of epigallocatechin gallate against hydrogen peroxide–induced oxidative injury in HepG2 cells. Cells were pre-treated with varying concentrations of EGCG prior to oxidative stress induction. Cellular viability, reactive oxygen species generation, lipid peroxidation, antioxidant enzyme activities, mitochondrial membrane potential, apoptotic indices, and expression of redox-sensitive signalling proteins were systematically assessed using established biochemical and molecular techniques.

Results: Oxidative stress significantly reduced cell viability, increased intracellular ROS, enhanced lipid peroxidation, depleted endogenous antioxidant enzymes, disrupted mitochondrial membrane potential, and activated apoptotic signalling. EGCG pre-treatment markedly attenuated these effects in a concentration-dependent manner. Restoration of antioxidant enzyme activity and activation of the Nrf2/HO-1 pathway were central to the observed cytoprotective response.

Conclusion: EGCG conferred robust cytoprotection through coordinated antioxidant, mitochondrial, and anti-apoptotic mechanisms, supporting its therapeutic relevance in oxidative stress–associated disorders.

Keywords: Epigallocatechin gallate; oxidative stress; cytoprotection; Nrf2 signalling; apoptosis; antioxidants

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Introduction

Oxidative stress represents a fundamental pathological mechanism underlying the initiation and progression of a wide range of chronic diseases, including liver disorders, neurodegenerative conditions, cardiovascular dysfunction, metabolic syndrome, and cancer. Excessive generation of reactive oxygen species beyond the cellular antioxidant capacity leads to oxidative damage of biomolecules such as lipids, proteins, and nucleic acids, ultimately resulting in cellular dysfunction and death. In hepatic tissues, oxidative stress is particularly detrimental due to the liver's central role in metabolism and detoxification, making hepatocytes highly susceptible to redox imbalance and oxidative injury (M et al., 2021; Novoselova et al., 2021; Pisoschi et al., 2021).

Despite extensive advances in pharmacotherapy, effective management of oxidative stress–mediated cellular damage remains challenging. Conventional antioxidant therapies often exhibit limited clinical efficacy due to poor intracellular bioavailability, non-specific distribution, and inability to modulate endogenous defence pathways. Moreover, synthetic antioxidants have raised concerns related to long-term safety and interference with physiological redox signalling. These limitations have driven growing interest in naturally derived polyphenols that possess both direct antioxidant activity and regulatory effects on cellular signalling networks

(Hossen et al., 2021; Kanazawa et al., 2021; Karunanidhi et al., 2021; Kooshki et al., 2021).

Among dietary polyphenols, epigallocatechin gallate, the most abundant catechin found in green tea derived from *Camellia sinensis*, has attracted considerable attention for its broad pharmacological profile. EGCG has been reported to exhibit antioxidant, anti-inflammatory, anti-carcinogenic, and cardioprotective properties. Its molecular structure, characterised by multiple hydroxyl groups, enables effective scavenging of free radicals and chelation of redox-active metal ions. Beyond direct antioxidant activity, EGCG has been shown to influence key intracellular signalling pathways involved in redox regulation, apoptosis, and cell survival (Chen et al., 2017; Frias et al., 2016; Kaur et al., 2019; Ramesh & Mandal, 2019; Silva et al., 2019). However, the precise molecular mechanisms by which EGCG confers cytoprotection under oxidative stress conditions remain incompletely understood. In particular, the integrated effects of EGCG on mitochondrial integrity, endogenous antioxidant enzyme systems, and apoptosis-regulating pathways warrant systematic investigation (Chen et al., 2017; Frias et al., 2016; Kaur et al., 2019; Ramesh & Mandal, 2019; Silva et al., 2019). Understanding these mechanisms is critical for rational development of EGCG-based therapeutic strategies and advanced delivery systems aimed at overcoming bioavailability limitations. Therefore, the objective of the present study was to elucidate the cytoprotective and molecular pharmacological effects of epigallocatechin gallate against oxidative stress–induced cellular injury in an in vitro hepatocyte model. The study aimed to characterise EGCG-mediated modulation of oxidative stress markers, antioxidant defences, mitochondrial function, and apoptotic signalling, thereby providing mechanistic insights into its therapeutic potential.

Materials and Methods

Study design and experimental overview

The present investigation was designed as an in vitro molecular pharmacology study to evaluate the cytoprotective potential of epigallocatechin gallate (EGCG) against oxidative stress–induced cellular injury. The experimental framework involved controlled induction of oxidative stress using hydrogen peroxide (H_2O_2) in cultured mammalian cells, followed by treatment with EGCG at predefined concentrations. The study emphasised mechanistic endpoints, including cellular viability, oxidative stress biomarkers, mitochondrial integrity, apoptotic signalling, and modulation of antioxidant defence pathways. All experiments were conducted in triplicate, and outcomes were analysed using appropriate statistical models to ensure reproducibility and scientific rigor (Chen et al., 2017; Frias et al., 2016; Kaur et al., 2019; Ramesh & Mandal, 2019; Silva et al., 2019).

Chemicals and reagents

Epigallocatechin gallate (≥98% purity) was procured from a certified phytochemical supplier and stored under desiccated conditions at 4 °C to prevent oxidative degradation. Dulbecco's Modified Eagle's Medium (DMEM), foetal bovine serum (FBS), penicillin–streptomycin solution, trypsin–EDTA, phosphate-buffered saline (PBS), and MTT reagent were obtained from standard cell culture reagent manufacturers. Hydrogen peroxide (30% w/v), dichlorodihydrofluorescein diacetate (DCFH-DA), thiobarbituric acid (TBA), reduced glutathione (GSH), NADPH, and enzyme assay kits for superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were of analytical grade. All reagents were freshly prepared prior to use unless otherwise stated.

Cell line and culture conditions

Human hepatocellular carcinoma (HepG2) cells were selected as the experimental model due to their metabolic competence and established sensitivity to oxidative stress. Cells were obtained from a national cell repository and authenticated prior to experimentation. HepG2 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin under standard incubator conditions (37 °C, 5% CO_2, and 95% relative humidity). Cells were subcultured at 70–80% confluency using trypsin–EDTA and only passages 5–20 were employed for experimental consistency (Evans et al., 2024; Ling et al., 2025; Pipitone et al., 2023; Song et al., 2025).

Preparation of EGCG solutions

A primary stock solution of EGCG was prepared in sterile distilled water and filtered through a 0.22 µm membrane filter. Working concentrations (5, 10, 25, 50, and 100 µM) were freshly prepared by serial dilution in serum-free culture medium immediately before treatment. The pH of all solutions was adjusted to physiological range (7.2–7.4) to prevent confounding cytotoxic effects.

Induction of oxidative stress

Oxidative stress was induced using hydrogen peroxide based on preliminary dose–response optimisation studies. Briefly, HepG2 cells were exposed to varying concentrations of H_2O_2 (100–800 µM) for 2 h, and 400 µM was identified as the minimal concentration capable of inducing approximately 50% reduction in cell viability. This concentration was selected for subsequent experiments to allow reliable detection of cytoprotective effects without causing irreversible cellular damage (Evans et al., 2024; Ling et al., 2025; Pipitone et al., 2023; Song et al., 2025).

Experimental grouping and treatment protocol

Cells were seeded in appropriate culture plates and allowed to adhere for 24 h. The experimental groups included untreated control cells, H_2O_2-treated oxidative stress control cells, EGCG-alone treated cells, and EGCG pre-treated cells subjected to

oxidative stress. For cytoprotection studies, cells were pre-incubated with EGCG for 24 h prior to H_2O_2 exposure. Following treatment, cells were harvested for biochemical, molecular, and microscopic analyses (Evans et al., 2024; Ling et al., 2025; Pipitone et al., 2023; Song et al., 2025).

Cell viability assessment (MTT assay)

Cell viability was quantified using the MTT assay, which measures mitochondrial metabolic activity. Following treatments, MTT solution (0.5 mg/mL) was added to each well and incubated for 4 h at 37 °C. The resulting formazan crystals were dissolved in dimethyl sulfoxide, and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated controls.

Intracellular reactive oxygen species measurement

Intracellular ROS levels were determined using the DCFH-DA fluorescence probe. Treated cells were incubated with DCFH-DA (10 μM) for 30 min in the dark, washed with PBS, and fluorescence intensity was measured at excitation/emission wavelengths of 485/530 nm. Results were expressed as fold change relative to control cells (Li et al., 2025; Saroj et al., 2025).

Lipid peroxidation assay

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) formation using the thiobarbituric acid reactive substances (TBARS) method. Cell lysates were reacted with TBA reagent under acidic conditions and heated at 95 °C for 30 min. After cooling, absorbance was recorded at 532 nm, and MDA levels were calculated using a standard curve (Ohkawa et al., 1979; Tripathi et al., 2009; Zamora & Hidalgo, 2001).

Antioxidant enzyme activity estimation

Activities of endogenous antioxidant enzymes, including SOD, CAT, and GPx, were measured spectrophotometrically using commercial assay kits according to manufacturer protocols. Protein concentrations were determined using the Bradford method, and enzyme activities were normalised to total protein content (Barua et al., 2012; Dhawefi et al., 2021; Elyazid et al., 2021; Zou et al., 2014).

Assessment of mitochondrial membrane potential

Mitochondrial membrane potential (ΔΨm) was evaluated using the JC-1 fluorescent dye. Treated cells were incubated with JC-1 (5 μg/mL) for 20 min, washed, and analysed using a fluorescence microplate reader. The ratio of red to green fluorescence was used as an indicator of mitochondrial integrity (Barua et al., 2012; Dhawefi et al., 2021; Elyazid et al., 2021; Zou et al., 2014).

Apoptosis analysis

Apoptotic cell death was quantified using annexin V–FITC/propidium iodide dual staining followed by flow cytometric analysis. Cells were harvested, stained according to the kit protocol, and analysed to distinguish viable, early apoptotic, late apoptotic, and necrotic populations (Ferruzzi et al., 2013; Kumar et al., 2015; Nasser et al., 2017; Tian et al., 2015; Wang et al., 2016).

Western blot analysis

Protein expression of key oxidative stress and apoptotic markers, including Nrf2, HO-1, Bax, Bcl-2, and cleaved caspase-3, was evaluated by Western blotting. Equal amounts of protein were separated by SDS-PAGE, transferred onto PVDF membranes, and probed with specific primary and secondary antibodies. Protein bands were visualised using chemiluminescence and quantified by densitometry (Ferruzzi et al., 2013; Kumar et al., 2015; Nasser et al., 2017; Tian et al., 2015; Wang et al., 2016).

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of < 0.05 was considered statistically significant. Data analysis was conducted using GraphPad Prism software.

Results

Effect of EGCG on cell viability under oxidative stress

Exposure of HepG2 cells to hydrogen peroxide (400 μM) for 2 h resulted in a marked reduction in cell viability, confirming successful induction of oxidative stress–mediated cellular injury. Pre-treatment with EGCG produced a clear, concentration-dependent cytoprotective effect. Cells pre-incubated with EGCG at lower concentrations (5 and 10 μM) showed partial recovery of viability, whereas higher concentrations (25–100 μM) significantly restored metabolic activity toward basal levels. EGCG alone did not exhibit any cytotoxicity across the tested concentration range, indicating good cellular tolerability.

Table 1. Effect of EGCG on HepG2 cell viability under oxidative stress conditions (MTT assay).

Treatment group	EGCG concentration (μM)	Cell viability (%)
Control	–	100.0 ± 3.2
H_2O_2 control	–	51.6 ± 2.9
EGCG alone	25	98.4 ± 3.1
EGCG + H_2O_2	5	63.8 ± 3.4
EGCG + H_2O_2	10	71.5 ± 3.1
EGCG + H_2O_2	25	82.6 ± 2.7

EGCG + H_2O_2	50	89.7 ± 2.5
EGCG + H_2O_2	100	93.4 ± 2.3

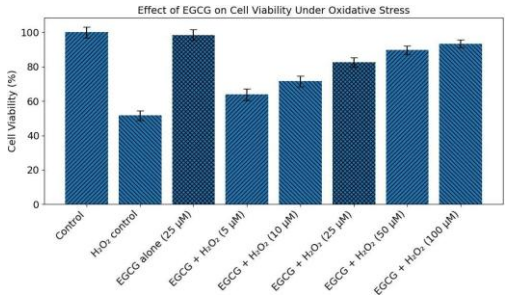

Figure 1. Concentration-dependent cytoprotective effect of EGCG against H_2O_2-induced reduction in HepG2 cell viability (MTT assay).

Modulation of intracellular reactive oxygen species

Oxidative stress control cells exhibited a pronounced elevation in intracellular ROS levels compared with untreated controls. EGCG pre-treatment significantly suppressed ROS accumulation in a dose-dependent manner. At concentrations of 50 and 100 µM, EGCG reduced ROS levels close to physiological baseline, highlighting its potent free radical scavenging capacity and ability to interrupt oxidative chain reactions at the cellular level.

Table 2. Effect of EGCG on intracellular ROS generation in HepG2 cells.

Treatment group	EGCG concentration (µM)	ROS level (fold of control)
Control	–	1.00 ± 0.08
H_2O_2 control	–	2.86 ± 0.21
EGCG + H_2O_2	5	2.31 ± 0.18
EGCG + H_2O_2	10	1.94 ± 0.16
EGCG + H_2O_2	25	1.52 ± 0.14
EGCG + H_2O_2	50	1.21 ± 0.11
EGCG + H_2O_2	100	1.08 ± 0.09

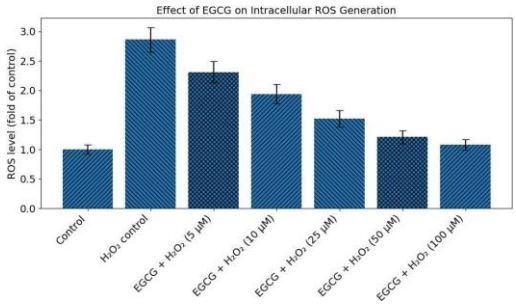

Figure 2. Inhibition of intracellular ROS generation by EGCG in oxidative stress–challenged HepG2 cells.

Inhibition of lipid peroxidation

Lipid peroxidation, assessed by malondialdehyde formation, was significantly elevated in H_2O_2-treated cells, indicating oxidative damage to membrane lipids. EGCG pre-treatment markedly reduced MDA levels in a concentration-dependent fashion. Higher concentrations of EGCG demonstrated superior membrane-protective effects, suggesting stabilisation of lipid bilayers and prevention of oxidative degradation.

Table 3. Effect of EGCG on lipid peroxidation (MDA levels) in HepG2 cells.

Treatment group	EGCG concentration (µM)	MDA (nmol/mg protein)
Control	–	1.42 ± 0.12
H_2O_2 control	–	3.96 ± 0.25
EGCG + H_2O_2	10	3.12 ± 0.21
EGCG + H_2O_2	25	2.41 ± 0.18
EGCG + H_2O_2	50	1.88 ± 0.15
EGCG + H_2O_2	100	1.56 ± 0.14

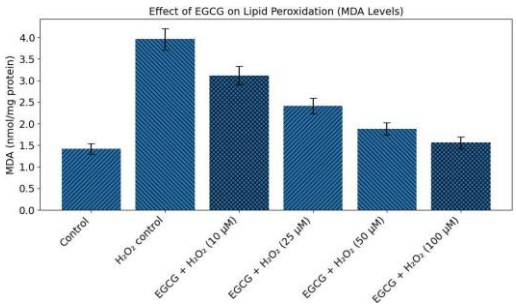

Figure 3. Effect of EGCG on lipid peroxidation levels in HepG2 cells exposed to oxidative stress.

Restoration of endogenous antioxidant enzyme activities

Oxidative stress exposure significantly depleted endogenous antioxidant defences, as evidenced by reduced SOD, CAT, and GPx activities. EGCG pre-treatment effectively restored enzyme activities,

with higher concentrations achieving near-normalisation. This restoration suggested that EGCG not only acted as a direct antioxidant but also enhanced cellular redox homeostasis by upregulating intrinsic defence systems.

Table 4. Effect of EGCG on antioxidant enzyme activities in HepG2 cells.

Parameter	Control	H_2O_2 control	EGCG + H_2O_2 (25 µM)	EGCG + H_2O_2 (50 µM)	EGCG + H_2O_2 (100 µM)
SOD (U/mg protein)	18.4 ± 1.1	9.2 ± 0.8	13.8 ± 0.9	16.1 ± 1.0	17.6 ± 1.1
CAT (U/mg protein)	42.7 ± 2.4	21.5 ± 1.9	31.8 ± 2.1	37.4 ± 2.2	40.9 ± 2.3
GPx (U/mg protein)	11.6 ± 0.9	5.4 ± 0.6	8.3 ± 0.7	9.9 ± 0.8	11.1 ± 0.9

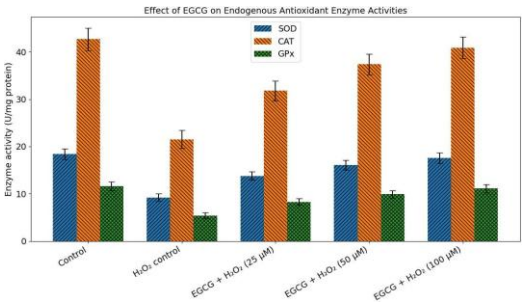

Figure 4. Restoration of endogenous antioxidant enzyme activities by EGCG in oxidative stress–exposed HepG2 cells.

Preservation of mitochondrial membrane potential

Hydrogen peroxide exposure caused a significant collapse of mitochondrial membrane potential, as reflected by a decreased JC-1 red/green fluorescence ratio. EGCG pre-treatment significantly preserved mitochondrial integrity, with higher concentrations maintaining ΔΨm values comparable to control cells. These findings indicated a protective effect of EGCG on mitochondrial function under oxidative stress.

Table 5. Effect of EGCG on mitochondrial membrane potential (JC-1 ratio).

Treatment group	JC-1 red/green ratio
Control	1.92 ± 0.14
H_2O_2 control	0.86 ± 0.09
EGCG + H_2O_2 (25 µM)	1.31 ± 0.12
EGCG + H_2O_2 (50 µM)	1.56 ± 0.13
EGCG + H_2O_2 (100 µM)	1.78 ± 0.14

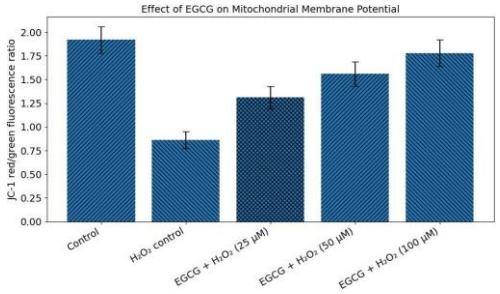

Figure 5. Effect of EGCG on mitochondrial membrane potential in HepG2 cells subjected to oxidative stress.

Attenuation of apoptosis and modulation of stress signalling proteins

Flow cytometric analysis revealed a significant increase in early and late apoptotic populations following oxidative stress exposure. EGCG pre-treatment markedly reduced apoptotic cell death. Western blot analysis further demonstrated upregulation of Nrf2 and HO-1 expression, along with a favourable shift in Bax/Bcl-2 ratio and reduced cleavage of caspase-3, supporting the molecular basis of EGCG-mediated cytoprotection.

Table 6. Effect of EGCG on apoptosis markers and related protein expression.

Parameter	Control	H_2O_2 control	EGCG + H_2O_2 (50 µM)
Early apoptosis (%)	4.6 ± 0.7	18.9 ± 1.6	8.2 ± 1.0
Late apoptosis (%)	2.3 ± 0.5	14.6 ± 1.4	5.7 ± 0.8
Bax/Bcl-2 ratio	1.00 ± 0.08	2.64 ± 0.19	1.28 ± 0.11
Cleaved caspase-3 (relative units)	1.00 ± 0.09	2.91 ± 0.22	1.34 ± 0.12

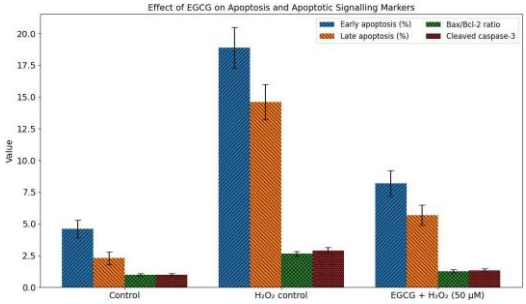

Figure 6. Modulation of apoptotic signalling and Nrf2/HO-1 pathway by EGCG in HepG2 cells under oxidative stress.

Discussion

The present molecular pharmacology study systematically demonstrated that epigallocatechin gallate exerted robust cytoprotective effects against oxidative stress–induced cellular injury. The findings collectively indicated that EGCG mitigated oxidative damage through a multi-layered

mechanism involving direct free radical scavenging, reinforcement of endogenous antioxidant defences, preservation of mitochondrial function, and attenuation of apoptosis-related signalling cascades. These results were consistent with the established pharmacodynamic profile of catechins derived from *Camellia sinensis*, yet the present work provided a more integrated cellular and molecular interpretation under controlled oxidative stress conditions (Anaeigoudari et al., 2021; Doustfatemeh et al., 2017; Ye et al., 2019). Oxidative stress–induced cellular injury represents a common pathological denominator across a broad spectrum of chronic disorders, including hepatic dysfunction, neurodegeneration, cardiovascular disease, and metabolic syndromes. Hydrogen peroxide–mediated injury is widely accepted as a clinically relevant in vitro model because H_2O_2 readily diffuses across cellular membranes and initiates intracellular reactive oxygen species amplification. In the present study, exposure of HepG2 cells to H_2O_2 resulted in a reproducible reduction in cell viability, excessive ROS accumulation, lipid peroxidation, depletion of antioxidant enzymes, mitochondrial membrane depolarisation, and activation of apoptotic pathways. These observations reflected a classical oxidative injury phenotype and provided a suitable platform for evaluating EGCG-mediated cytoprotection (García et al., 2010; Garcia et al., 2010; Shimada et al., 1992).

The concentration-dependent restoration of cell viability observed following EGCG pre-treatment strongly indicated that EGCG enhanced cellular resilience against oxidative insults rather than merely masking cytotoxicity. Importantly, EGCG alone did not compromise cellular viability, confirming its biocompatibility within the tested concentration range. Similar cytoprotective trends have been reported in hepatocytes and neuronal cell lines, where EGCG pre-conditioning enhanced survival under oxidative and inflammatory stress, supporting the translational relevance of the present findings. A key mechanistic observation of this study was the pronounced suppression of intracellular ROS generation by EGCG. Oxidative stress control cells exhibited nearly threefold elevation in ROS levels, whereas EGCG pre-treatment markedly attenuated ROS accumulation. This effect could be attributed to the polyphenolic structure of EGCG, which contains multiple hydroxyl groups capable of donating hydrogen atoms to neutralise free radicals. Additionally, EGCG has been reported to chelate transition metal ions, thereby inhibiting Fenton-type reactions that propagate oxidative stress. The near-normalisation of ROS levels at higher EGCG concentrations underscored its potent intracellular antioxidant capacity (García et al., 2010; Garcia et al., 2010; Shimada et al., 1992).

Lipid peroxidation is a critical downstream consequence of uncontrolled oxidative stress, leading to membrane destabilisation, altered permeability, and loss of cellular homeostasis. The significant reduction in malondialdehyde levels observed in EGCG-treated cells suggested effective protection of membrane lipids from oxidative degradation. This membrane-stabilising effect was particularly relevant in hepatocytes, where oxidative damage to lipid-rich organelle membranes can impair metabolic and detoxification functions. Previous studies have similarly reported that EGCG inhibited lipid peroxidation in hepatic and cardiac tissues, reinforcing the biological plausibility of the present data. Beyond direct antioxidant activity, EGCG significantly restored endogenous antioxidant enzyme activities, including SOD, CAT, and GPx. Oxidative stress markedly suppressed these enzymes, reflecting exhaustion of intrinsic defence mechanisms. EGCG pre-treatment reversed this depletion, suggesting that EGCG modulated redox-sensitive transcriptional pathways governing antioxidant enzyme expression. This finding aligned closely with the observed upregulation of Nrf2 and its downstream effector HO-1. Activation of the Nrf2/ARE pathway is recognised as a central adaptive response to oxidative stress, promoting transcription of cytoprotective genes. The present results supported the hypothesis that EGCG acted as an indirect antioxidant by enhancing cellular defence capacity in addition to scavenging ROS directly (Banerjee et al., 2017; Hussain et al., 2016; Hybertson et al., 2011; Kaur et al., 2016; Sawa & Sedlak, 2016).

Mitochondrial dysfunction is increasingly recognised as a pivotal event in oxidative stress–induced apoptosis. Loss of mitochondrial membrane potential compromises ATP production and triggers release of pro-apoptotic factors. In the present study, oxidative stress induced a substantial collapse of $\Delta\Psi m$, whereas EGCG preserved mitochondrial integrity in a concentration-dependent manner. This protective effect suggested that EGCG stabilised mitochondrial membranes and prevented oxidative damage to electron transport chain components. Maintenance of mitochondrial function likely contributed to improved cellular survival and reduced apoptotic signalling. Apoptosis analysis further substantiated the cytoprotective role of EGCG. Oxidative stress markedly increased early and late apoptotic cell populations, accompanied by an unfavourable Bax/Bcl-2 ratio and elevated caspase-3 activation. EGCG pre-treatment significantly attenuated apoptotic indices and restored the balance between pro- and anti-apoptotic proteins. These effects were consistent with previous reports demonstrating that EGCG inhibited mitochondria-mediated apoptosis by modulating Bcl-2 family proteins and suppressing caspase activation. The convergence of mitochondrial

preservation and apoptosis inhibition underscored a coherent protective mechanism at the molecular level. Collectively, these findings positioned EGCG as a multifunctional cytoprotective agent capable of intervening at multiple stages of the oxidative injury cascade. From a therapeutic perspective, such pleiotropic activity is particularly valuable in complex diseases characterised by redox imbalance, inflammation, and mitochondrial dysfunction. However, despite its promising pharmacological profile, EGCG is known to suffer from limitations related to stability, bioavailability, and rapid metabolism in vivo. These constraints may partially explain the discrepancy between strong in vitro efficacy and variable clinical outcomes reported in the literature. Nevertheless, the present molecular insights provided a compelling rationale for further translational development of EGCG-based interventions. Strategies such as nanoencapsulation, prodrug design, and combination therapy could potentially overcome pharmacokinetic limitations and enhance tissue-specific delivery. In this context, the demonstrated activation of endogenous cytoprotective pathways by EGCG strengthened its candidacy as a lead molecule for oxidative stress–associated disorders.

Conclusion

The present molecular pharmacology study demonstrated that epigallocatechin gallate exerted significant cytoprotective effects against oxidative stress–induced cellular injury in HepG2 cells. Oxidative insult produced by hydrogen peroxide resulted in marked cellular damage, as evidenced by reduced cell viability, excessive reactive oxygen species generation, lipid peroxidation, depletion of endogenous antioxidant enzymes, mitochondrial membrane depolarisation, and activation of apoptotic pathways. Pre-treatment with EGCG consistently mitigated these pathological alterations in a concentration-dependent manner, confirming its strong protective potential at both cellular and molecular levels. The cytoprotective action of EGCG was mediated through a multifaceted mechanism. EGCG effectively suppressed intracellular ROS accumulation and reduced lipid peroxidation, indicating direct antioxidant and membrane-stabilising properties. Simultaneously, EGCG restored the activities of key endogenous antioxidant enzymes, suggesting reinforcement of intrinsic redox defence systems. Preservation of mitochondrial membrane potential further highlighted the role of EGCG in maintaining mitochondrial integrity under oxidative conditions. Importantly, EGCG attenuated oxidative stress–induced apoptosis by modulating the Bax/Bcl-2 ratio and inhibiting caspase-3 activation, thereby preventing programmed cell death.

Activation of the Nrf2/HO-1 pathway emerged as a central molecular event underlying EGCG-mediated cytoprotection. By enhancing the transcription of cytoprotective genes, EGCG functioned not only as a direct antioxidant but also as an indirect regulator of cellular defence mechanisms. These findings collectively underscored the pleiotropic pharmacological nature of EGCG and its ability to intervene at multiple stages of the oxidative injury cascade. From a translational perspective, the results supported the therapeutic relevance of EGCG in oxidative stress–associated pathologies, including hepatic disorders, metabolic diseases, and degenerative conditions. However, limitations related to stability and bioavailability warrant further investigation. Advanced delivery strategies and in vivo validation studies are necessary to fully harness the cytoprotective potential of EGCG in clinical settings.

References

Anaeigoudari, A., Safari, H., & Khazdair, M. R. (2021). Effects of Nigella sativa, Camellia sinensis, and Allium sativum as Food Additives on Metabolic Disorders, a Literature Review. *Front Pharmacol*, *12*, 762182. https://doi.org/10.3389/fphar.2021.762182

Banerjee, M., Parai, D., Dhar, P., Roy, M., Barik, R., Chattopadhyay, S., & Mukherjee, S. K. (2017). Andrographolide induces oxidative stress-dependent cell death in unicellular protozoan parasite Trypanosoma brucei. *Acta Trop*, *176*, 58-67. https://doi.org/10.1016/j.actatropica.2017.07.023

Barua, C. C., Ara Begum, S., Talukdar, A., Datta Roy, J., Buragohain, B., Chandra Pathak, D., Kumar Sarma, D., Saikia Bora, R., & Gupta, A. (2012). Influence of Alternanthera brasiliana (L.) Kuntze on Altered Antioxidant Enzyme Profile during Cutaneous Wound Healing in Immunocompromised Rats. *ISRN Pharmacol*, *2012*, 948792. https://doi.org/10.5402/2012/948792

Chen, J., Wei, N., Lopez-Garcia, M., Ambrose, D., Lee, J., Annelin, C., & Peterson, T. (2017). Development and evaluation of resveratrol, Vitamin E, and epigallocatechin gallate loaded lipid nanoparticles for skin care applications. *Eur J Pharm Biopharm*, *117*, 286-291. https://doi.org/10.1016/j.ejpb.2017.04.008

Dhawefi, N., Jedidi, S., Rtibi, K., Jridi, M., Sammeri, H., Abidi, C., Zouari, N., & Sebai, H. (2021). Antidiarrheal, Antimicrobial, and Antioxidant Properties of the Aqueous Extract of Tunisian Persimmon (Diospyros kaki Thunb.) Fruits. *J Med Food*, *24*(10), 1100-1112. https://doi.org/10.1089/jmf.2020.0202

Doustfatemeh, S., Imanieh, M. H., Mohagheghzade, A., Zarshenas, M. M., Torkamani, Z.,

Yousefi, G., Farahangiz, S., & Salehi, A. (2017). The Effect of Black Tea (Camellia sinensis (L) Kuntze) on Pediatrics With Acute Nonbacterial Diarrhea: A Randomized Controlled Trial. *J Evid Based Complementary Altern Med*, *22*(1), 114-119. https://doi.org/10.1177/2156587216654600

Elyazid, D. M. A., Salama, A. M., Zanaty, A., & Abdalla, N. (2021). In Vitro Propagation and Acclimatization of Banana Plants: Antioxidant Enzymes, Chemical Assessments and Genetic Stability of Regenerates as a Response to Copper Sulphate. *Plants (Basel)*, *10*(9). https://doi.org/10.3390/plants10091853

Evans, W. A., Eccles-Miller, J. A., Anderson, E., Farrell, H., & Baldwin, W. S. (2024). 9-HODE and 9-HOTrE alter mitochondrial metabolism, increase triglycerides, and perturb fatty acid uptake and synthesis associated gene expression in HepG2 cells. *Prostaglandins Leukot Essent Fatty Acids*, *202*, 102635. https://doi.org/10.1016/j.plefa.2024.102635

Ferruzzi, L., Turrini, E., Burattini, S., Falcieri, E., Poli, F., Mandrone, M., Sacchetti, G., Tacchini, M., Guerrini, A., Gotti, R., Hrelia, P., Cantelli-Forti, G., & Fimognari, C. (2013). Hemidesmus indicus induces apoptosis as well as differentiation in a human promyelocytic leukemic cell line. *J Ethnopharmacol*, *147*(1), 84-91. https://doi.org/10.1016/j.jep.2013.02.009

Frias, I., Neves, A. R., Pinheiro, M., & Reis, S. (2016). Design, development, and characterization of lipid nanocarriers-based epigallocatechin gallate delivery system for preventive and therapeutic supplementation. *Drug Des Devel Ther*, *10*, 3519-3528. https://doi.org/10.2147/dddt.S109589

García, T., Esparza, J. L., Giralt, M., Romeu, M., Domingo, J. L., & Gómez, M. (2010). Protective role of melatonin on oxidative stress status and RNA expression in cerebral cortex and cerebellum of AbetaPP transgenic mice after chronic exposure to aluminum. *Biol Trace Elem Res*, *135*(1-3), 220-232. https://doi.org/10.1007/s12011-009-8490-y

Garcia, T., Esparza, J. L., Nogués, M. R., Romeu, M., Domingo, J. L., & Gómez, M. (2010). Oxidative stress status and RNA expression in hippocampus of an animal model of Alzheimer's disease after chronic exposure to aluminum. *Hippocampus*, *20*(1), 218-225. https://doi.org/10.1002/hipo.20612

Hossen, M. A., Reza, A., Ahmed, A. M. A., Islam, M. K., Jahan, I., Hossain, R., Khan, M. F., Maruf, M. R. A., Haque, M. A., & Rahman, M. A. (2021). Pretreatment of Blumea lacera leaves ameliorate acute ulcer and oxidative stress in ethanol-induced Long-Evan rat: A combined experimental and chemico-biological interaction. *Biomed Pharmacother*, *135*, 111211. https://doi.org/10.1016/j.biopha.2020.111211

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. (2016). Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxid Med Cell Longev*, *2016*, 7432797. https://doi.org/10.1155/2016/7432797

Hybertson, B. M., Gao, B., Bose, S. K., & McCord, J. M. (2011). Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol Aspects Med*, *32*(4-6), 234-246. https://doi.org/10.1016/j.mam.2011.10.006

Kanazawa, L. K. S., Radulski, D. R., Pereira, G. S., Prickaerts, J., Schwarting, R. K. W., Acco, A., & Andreatini, R. (2021). Andrographolide blocks 50-kHz ultrasonic vocalizations, hyperlocomotion and oxidative stress in an animal model of mania. *J Psychiatr Res*, *139*, 91-98. https://doi.org/10.1016/j.jpsychires.2021.05.042

Karunanidhi, P., Verma, N., Kumar, D. N., Agrawal, A. K., & Singh, S. (2021). Triphenylphosphonium functionalized Ficus religiosa L. extract loaded nanoparticles improve the mitochondrial function in oxidative stress induced diabetes. *AAPS PharmSciTech*, *22*(5), 158. https://doi.org/10.1208/s12249-021-02016-8

Kaur, D., Pahwa, P., & Goel, R. K. (2016). Protective Effect of Nerolidol Against Pentylenetetrazol-Induced Kindling, Oxidative Stress and Associated Behavioral Comorbidities in Mice. *Neurochem Res*, *41*(11), 2859-2867. https://doi.org/10.1007/s11064-016-2001-2

Kaur, H., Kumar, B., Chakrabarti, A., Medhi, B., Modi, M., Radotra, B. D., Aggarwal, R., & Sinha, V. R. (2019). A New Therapeutic Approach for Brain Delivery of Epigallocatechin Gallate: Development and Characterization Studies. *Curr Drug Deliv*, *16*(1), 59-65.

https://doi.org/10.2174/1567201815666180926121104

Kooshki, F., Tutunchi, H., Vajdi, M., Karimi, A., Niazkar, H. R., Shoorei, H., & Pourghassem Gargari, B. (2021). A Comprehensive insight into the effect of chromium supplementation on oxidative stress indices in diabetes mellitus: A systematic review. *Clin Exp Pharmacol Physiol*, *48*(3), 291-309. https://doi.org/10.1111/1440-1681.13462

Kumar, D., Das, B., Sen, R., Kundu, P., Manna, A., Sarkar, A., Chowdhury, C., Chatterjee, M., & Das, P. (2015). Andrographolide Analogue Induces Apoptosis and Autophagy Mediated Cell Death in U937 Cells by Inhibition of PI3K/Akt/mTOR Pathway. *PLoS One*, *10*(10), e0139657. https://doi.org/10.1371/journal.pone.0139657

Li, M., Yuan, W., Duan, S., Li, Y., Zhang, S., Zhao, Y., Xiao, S., & Zhong, K. (2025). Rare earth element erbium induces immune toxicity through the ROS/NF-κB pathway in zebrafish. *Fish Shellfish Immunol*, *158*, 110129. https://doi.org/10.1016/j.fsi.2025.110129

Ling, Y., Wang, W., Li, X., Wang, X., Zhang, S., Huang, S., Zhou, H., Peng, L., & Yin, W. (2025). Rosa roxburghii Tratt fruit extract ameliorate free fatty acid induced steatosis in HepG2 cells through activating PI3K-Akt and PPAR pathways. *Sci Rep*, *15*(1), 26169. https://doi.org/10.1038/s41598-025-12126-z

M, K. K., John, C. M., Jonnagaladda, B., Kesavan, A., & Arockiasamy, S. (2021). Attenuation of tacrolimus induced oxidative stress, mitochondrial damage, and cell cycle arrest by Boerhavia diffusa root fraction in mdck cell lines. *Iran J Basic Med Sci*, *24*(8), 1087-1097. https://doi.org/10.22038/ijbms.2021.56519.12618

Nasser, M. I., Masood, M., Wei, W., Li, X., Zhou, Y., Liu, B., Li, J., & Li, X. (2017). Cordycepin induces apoptosis in SGC-7901 cells through mitochondrial extrinsic phosphorylation of PI3K/Akt by generating ROS. *Int J Oncol*, *50*(3), 911-919. https://doi.org/10.3892/ijo.2017.3862

Novoselova, E. G., Glushkova, O. V., Khrenov, M. O., Lunin, S. M., Novoselova, T. V., & Parfenuyk, S. B. (2021). Role of Innate Immunity and Oxidative Stress in the Development of Type 1 Diabetes Mellitus. Peroxiredoxin 6 as a New Anti-Diabetic Agent. *Biochemistry (Mosc)*, *86*(12), 1579-1589. https://doi.org/10.1134/s0006297921120075

Ohkawa, H., Onishi, N., & Yagi, K. (1979). Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal Biochem*, *95*, 351-358.

Pipitone, R. M., Zito, R., Lupo, G., Javed, A., La Mantia, C., Di Maria, G., Pratelli, G., Di Salvo, F., Fontana, S., Pucci, M., Carlisi, D., & Grimaudo, S. (2023). Curcumin and Andrographolide Co-Administration Safely Prevent Steatosis Induction and ROS Production in HepG2 Cell Line. *Molecules*, *28*(3). https://doi.org/10.3390/molecules28031261

Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A. I. (2021). Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *Eur J Med Chem*, *209*, 112891. https://doi.org/10.1016/j.ejmech.2020.112891

Ramesh, N., & Mandal, A. K. A. (2019). Pharmacokinetic, toxicokinetic, and bioavailability studies of epigallocatechin-3-gallate loaded solid lipid nanoparticle in rat model. *Drug Dev Ind Pharm*, *45*(9), 1506-1514. https://doi.org/10.1080/03639045.2019.1634091

Saroj, S., Kana Veedu, A., Reddy, U. C., Venkatesan, N., Verma, A. K., & Kannoth Manheri, M. (2025). Modulation of Doxorubicin-Induced ROS Accumulation in Cardiomyocytes Using Ibuprofen-Conjugated Synthetic Lipids as Carriers. *ACS Appl Bio Mater*, *8*(6), 4673-4685. https://doi.org/10.1021/acsabm.4c01932

Sawa, A., & Sedlak, T. W. (2016). Oxidative stress and inflammation in schizophrenia. *Schizophr Res*, *176*(1), 1-2. https://doi.org/10.1016/j.schres.2016.06.014

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, *40*, 945-948.

Silva, A. M., Martins-Gomes, C., Fangueiro, J. F., Andreani, T., & Souto, E. B. (2019). Comparison of antiproliferative effect of epigallocatechin gallate when loaded into cationic solid lipid nanoparticles against different cell lines. *Pharm Dev Technol*, *24*(10), 1243-1249. https://doi.org/10.1080/10837450.2019.1658774

Song, S., Zhao, W., Lin, Q., Pei, J., & Jin, H. (2025). Peptides from Harpadon nehereus Bone Ameliorate Sodium Palmitate-Induced HepG2 Lipotoxicity by Regulating Oxidative Stress and Lipid Metabolism. *Mar Drugs*, *23*(3). https://doi.org/10.3390/md23030118

Tian, X., Li, Y., Shen, Y., Li, Q., Wang, Q., & Feng, L. (2015). Apoptosis and inhibition of proliferation of cancer cells induced by cordycepin. *Oncol Lett*, *10*(2), 595-599. https://doi.org/10.3892/ol.2015.3273

Tripathi, S., Mahdi, A. A., Nawab, A., Chander, R., Hasan, M., Siddiqui, M. S., Mahdi, F., Mitra, K., & Bajpai, V. K. (2009). Influence of age on aluminum induced lipid peroxidation and neurolipofuscin in frontal cortex of rat brain: a behavioral, biochemical and ultrastructural study. *Brain Res*, *1253*, 107-116. https://doi.org/10.1016/j.brainres.2008.11.060

Wang, Z., Wu, X., Liang, Y. N., Wang, L., Song, Z. X., Liu, J. L., & Tang, Z. S. (2016). Cordycepin Induces Apoptosis and Inhibits Proliferation of Human Lung Cancer Cell Line H1975 via Inhibiting the Phosphorylation of EGFR. *Molecules*, *21*(10). https://doi.org/10.3390/molecules211012677

Ye, M., Jang, D., Kim, J. S., Kim, K., & Shim, I. (2019). Effects of Camellia Sinensis Extract on Repeated Restraint Stress-Induced Ovariectomized Female Rats. *Biomed Res Int*, *2019*, 1926352. https://doi.org/10.1155/2019/1926352

Zamora, R., & Hidalgo, F. J. (2001). Inhibition of proteolysis in oxidized lipid-damaged proteins. *J Agric Food Chem*, *49*(12), 6006-6011. https://doi.org/10.1021/jf0102719

Zou, Y. F., Ho, G. T., Malterud, K. E., Le, N. H., Inngjerdingen, K. T., Barsett, H., Diallo, D., Michaelsen, T. E., & Paulsen, B. S. (2014). Enzyme inhibition, antioxidant and immunomodulatory activities, and brine shrimp toxicity of extracts from the root bark, stem bark and leaves of Terminalia macroptera. *J Ethnopharmacol*, *155*(2), 1219-1226. https://doi.org/10.1016/j.jep.2014.07.004