RESEARCH PAPER

In Vitro Assessment of the Cytoprotective Activity of Syzygium cumini Seed Extract on RIN-5F Pancreatic β-Cell Lines and HUH-7 Hepatocellular Carcinoma Cell Lines

Anshuman Prajapati1, Priyanka Prajapati2, Ajay Kumar Gupta1*

1Chhatrapati Sahu Ji Maharaj University, Kanpur, Uttar Pradesh, 208024, India

2Krishna Institute of Pharmacy and Sciences, Kanpur, Uttar Pradesh, 209217, India

Corresponding Author: Ajay Kumar Gupta

Abstract

Background

Oxidative stress is a critical mediator of pancreatic β-cell dysfunction and hepatic cellular injury in diabetes and related metabolic disorders. Pancreatic β-cells are particularly susceptible to oxidative damage due to limited intrinsic antioxidant capacity, while hepatic cells are central to metabolic regulation and redox homeostasis. Natural phytochemicals rich in polyphenols have gained attention for their antioxidant and cytoprotective potential. *Syzygium cumini* seeds, traditionally used in glycemic management, contain bioactive compounds with established free radical scavenging properties. The present study evaluated the in vitro cytoprotective activity of *Syzygium cumini* seed extract (SCSE) in pancreatic and hepatic cell models subjected to oxidative stress.

Methods

Ethanolic extract of *Syzygium cumini* seeds was prepared and subjected to qualitative and quantitative phytochemical analysis. RIN-5F pancreatic β-cell lines and HUH-7 hepatocellular carcinoma cell lines were cultured under standard conditions. Oxidative stress was induced using hydrogen peroxide. Cytoprotective effects were assessed using MTT assay. Intracellular reactive oxygen species (ROS), lipid peroxidation (malondialdehyde levels), and antioxidant defense markers including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were evaluated. Statistical significance was determined using one-way ANOVA.

Results

Hydrogen peroxide exposure significantly reduced cell viability and increased oxidative stress markers in both cell lines. Pretreatment with SCSE produced a dose-dependent restoration of viability and significantly reduced ROS generation and lipid peroxidation. Antioxidant enzyme activities and GSH levels were markedly improved following extract treatment. The protective effect was more pronounced in RIN-5F β-cells compared to HUH-7 cells, indicating differential cellular responsiveness.

Conclusion

Syzygium cumini seed extract demonstrates significant cytoprotective and antioxidant activity in pancreatic and hepatic cellular models. These findings provide mechanistic support for its therapeutic potential in oxidative stress–associated metabolic disorders and warrant further in vivo and translational investigations.

Keywords

Syzygium cumini, Cytoprotection, Oxidative stress, Pancreatic β-cells, Hepatocellular carcinoma, Antioxidant activity

How to cite this article: Prajapati A, Prajapati P, Gupta AK. In Vitro Assessment of the Cytoprotective Activity of Syzygium cumini Seed Extract on RIN-5F Pancreatic β-Cell Lines and HUH-7 Hepatocellular Carcinoma Cell Lines. Int J Drug Deliv Technol. 2026;16(9s): 85-97; DOI: 10.25258/ijddt.16.9s.9

Introduction

Diabetes mellitus and chronic liver diseases continue to pose major global health challenges, contributing substantially to morbidity, mortality, and healthcare burden worldwide. A unifying pathogenic feature underlying these disorders is oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the capacity of endogenous antioxidant defense systems to neutralize them. Persistent oxidative stress disrupts cellular

homeostasis, damages macromolecules including lipids, proteins, and DNA, and contributes to metabolic dysfunction in multiple organs. Increasing evidence indicates that oxidative stress is not merely a consequence of metabolic disease but a critical mechanistic driver of disease progression, particularly in pancreatic β-cells and hepatic tissues [1,2].

Pancreatic β-cells play a central role in maintaining glucose homeostasis through the synthesis and secretion of insulin in response to metabolic stimuli. However, these specialized cells possess relatively low levels of antioxidant enzymes, including catalase, glutathione peroxidase, and superoxide dismutase, rendering them particularly susceptible to oxidative damage [3]. Under conditions of chronic hyperglycemia and metabolic overload, excessive ROS generation can impair mitochondrial function, disrupt insulin gene expression, and trigger apoptotic pathways leading to progressive β-cell dysfunction and loss. Oxidative stress has therefore emerged as a pivotal factor in the pathogenesis of diabetes, contributing to impaired insulin secretion and deterioration of glycemic control [4,5]. Moreover, ROS-mediated damage has been shown to alter key transcription factors involved in β-cell identity and insulin production, including PDX-1 and MAFA, further exacerbating β-cell failure during metabolic stress [6].

In addition to pancreatic dysfunction, oxidative stress also plays a critical role in hepatic pathophysiology. The liver serves as a central metabolic organ responsible for glucose and lipid metabolism, detoxification, and regulation of systemic redox balance. Hepatic cells are constantly exposed to reactive oxygen species generated during mitochondrial respiration, xenobiotic metabolism, and inflammatory processes. When ROS production exceeds the detoxifying capacity of hepatic antioxidant systems, oxidative injury can occur, contributing to liver diseases such as non-alcoholic fatty liver disease, fibrosis, and hepatocellular carcinoma [7,8]. In hepatic carcinoma cells, redox signaling pathways influence cell proliferation, apoptosis, and metabolic adaptation, highlighting the complex role of oxidative stress in tumor biology. Consequently, modulation of redox homeostasis has been proposed as a promising strategy for preventing oxidative damage and improving therapeutic outcomes in liver diseases [9,10].

Because oxidative stress contributes to cellular injury across multiple tissues, considerable research has focused on identifying therapeutic agents capable of restoring redox balance. Natural phytochemicals derived from medicinal plants have attracted increasing attention due to their antioxidant, anti-inflammatory, and metabolic regulatory properties. Many plant-derived compounds contain phenolics, flavonoids, tannins, and other bioactive constituents that can scavenge free radicals, chelate transition metals, and enhance endogenous antioxidant defense mechanisms [11]. In addition to their direct antioxidant effects, these phytochemicals can modulate intracellular signaling pathways associated with oxidative stress, including pathways involved in mitochondrial protection, inflammatory regulation, and cellular survival [12]. Such multifunctional actions make plant-derived antioxidants promising candidates for mitigating oxidative stress–related metabolic disorders.

Among medicinal plants with recognized therapeutic potential, **Syzygium cumini (L.) Skeels**, commonly known as Jamun or Indian blackberry, has been extensively used in traditional medicine for the management of diabetes and other metabolic conditions. Various parts of the plant including fruits, leaves, bark, and seeds contain a wide spectrum of phytochemicals with demonstrated pharmacological activity. In particular, the seeds of *Syzygium cumini* are rich in polyphenolic compounds such as flavonoids, ellagic acid, gallic acid, tannins, and anthocyanins, which contribute to their antioxidant and antidiabetic properties [13,14]. These bioactive molecules have been reported to exert diverse biological effects, including free radical scavenging, inhibition of lipid peroxidation, and modulation of carbohydrate metabolism.

Recent phytochemical and pharmacological studies have further highlighted the potential of *Syzygium cumini* seeds as a source of natural therapeutic agents. Investigations have demonstrated that jamun seed extracts possess strong antioxidant activity and can reduce oxidative stress in various experimental models [15]. The beneficial effects of these extracts have been attributed to their high phenolic and flavonoid content, which can neutralize reactive oxygen species and enhance endogenous antioxidant enzyme activity. In addition to antioxidant activity, jamun seed constituents have been reported to exhibit anti-inflammatory, hepatoprotective, and glucose-lowering effects, suggesting their potential relevance in the management of metabolic disorders and liver diseases [16].

Advances in analytical techniques have facilitated more precise characterization of the phytochemical composition of *Syzygium cumini* seeds. Modern

extraction and analytical approaches have identified numerous secondary metabolites, including hydrolysable tannins, terpenoids, alkaloids, and phenolic acids that contribute to the plant's pharmacological properties [17]. These compounds not only demonstrate strong radical-scavenging activity but may also regulate intracellular antioxidant pathways involved in maintaining cellular redox balance. Such findings provide a scientific basis for exploring the cytoprotective potential of jamun seed extracts in cellular models of oxidative stress.

Beyond their traditional medicinal use, plant-derived antioxidants have increasingly been investigated in cell culture models to understand their mechanisms of action at the molecular level. Pancreatic β-cell lines and hepatic carcinoma cell lines are commonly employed experimental models to evaluate oxidative stress responses and antioxidant interventions. These in vitro systems enable controlled investigation of cellular viability, reactive oxygen species generation, lipid peroxidation, and endogenous antioxidant enzyme activity following exposure to oxidative insults [18]. Hydrogen peroxide is frequently used as a model oxidant in such studies because it readily penetrates cellular membranes and generates secondary reactive species capable of inducing oxidative injury.

Despite growing evidence supporting the antioxidant potential of *Syzygium cumini*, limited studies have examined its cytoprotective effects simultaneously in pancreatic and hepatic cellular systems under oxidative stress conditions. Considering the central role of oxidative stress in both diabetes and liver disease, comparative evaluation of the protective effects of plant-derived antioxidants in different cell types may provide valuable mechanistic insights into their therapeutic relevance. Investigating how these extracts influence redox balance in insulin-secreting cells and hepatic carcinoma cells may also help clarify whether their biological effects are universally protective or context-dependent [19].

Therefore, the present study was designed to investigate the in vitro cytoprotective activity of *Syzygium cumini* seed extract in RIN-5F pancreatic β-cell lines and HUH-7 hepatocellular carcinoma cell lines exposed to hydrogen peroxide–induced oxidative stress. By evaluating cell viability, intracellular reactive oxygen species generation, lipid peroxidation, and antioxidant enzyme activity, this study aims to elucidate the potential antioxidant mechanisms underlying the protective effects of the extract. Understanding these mechanisms may contribute to the development of plant-based therapeutic strategies targeting oxidative stress in metabolic and hepatic disorders [20].

Objectives

The primary objective of the present study was to evaluate the in vitro cytoprotective potential of *Syzygium cumini* seed extract against hydrogen peroxide–induced oxidative stress in RIN-5F pancreatic β-cell lines and HUH-7 hepatocellular carcinoma cell lines. The study aimed to determine whether pretreatment with the extract could preserve cellular viability and attenuate oxidative damage under stress conditions.

A secondary objective was to investigate the antioxidant mechanisms underlying the observed cytoprotective effects by assessing intracellular reactive oxygen species (ROS) levels, lipid peroxidation (MDA), and endogenous antioxidant defense markers, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). These evaluations were intended to elucidate the potential role of phytoconstituents present in *Syzygium cumini* seeds in mitigating oxidative injury in pancreatic and hepatic cellular models.

Methodology

Study Design

This investigation was conducted as a controlled in vitro experimental study designed to evaluate the cytoprotective and antioxidant effects of *Syzygium cumini* seed extract on pancreatic and hepatic cell lines under induced oxidative stress conditions. The methodological framework was structured in accordance with principles analogous to the STROBE criteria, adapted for laboratory-based cellular research, ensuring clarity in study design, exposure definition, outcome assessment, and statistical analysis.

Study Setting

All experimental procedures were carried out in a controlled cell culture laboratory environment under standardized aseptic conditions. Temperature, humidity, and carbon dioxide concentration were maintained consistently to ensure optimal cellular growth and reproducibility of results.

Plant Material Procurement and Authentication

Mature seeds of *Syzygium cumini* were procured from a verified botanical source. The plant material underwent taxonomic authentication by a qualified botanist. Following authentication, seeds were washed, shade-dried at room temperature to prevent degradation of thermolabile constituents, and mechanically powdered to obtain a uniform coarse powder for extraction.

Preparation of Seed Extract

The powdered seed material was subjected to solvent extraction using 70% ethanol in a Soxhlet apparatus. Extraction was continued until complete exhaustion of phytoconstituents was achieved. The extract was concentrated under reduced pressure using a rotary evaporator and subsequently dried to obtain a semi-solid mass. The dried extract was stored in airtight containers at 4°C until further experimental use. Working concentrations were prepared freshly in dimethyl sulfoxide and diluted in culture medium to achieve the required test concentrations, ensuring that solvent concentration remained non-cytotoxic.

Preliminary Phytochemical Analysis

Qualitative phytochemical screening was performed to identify major classes of secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, and saponins. Quantitative estimation of total phenolic content and total flavonoid content was carried out using spectrophotometric methods. Results were expressed in standard equivalent units to assess the antioxidant potential of the extract.

Cell Lines and Culture Conditions

RIN-5F pancreatic β-cell lines and HUH-7 hepatocellular carcinoma cell lines were obtained from a certified cell repository. Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum and antibiotic solution. Cultures were maintained in a humidified incubator at 37°C with 5% CO_2. Cells were subcultured upon reaching 70–80% confluency using standard trypsinization procedures to ensure uniform growth kinetics.

Exposure Assessment and Experimental Groups

Oxidative stress was induced using hydrogen peroxide at a standardized concentration determined through preliminary dose optimization experiments. Cells were divided into defined experimental groups including untreated control, oxidative stress control, and extract-treated groups exposed to graded concentrations of *Syzygium cumini* seed extract prior to oxidative insult. Pretreatment duration and exposure time were standardized to ensure comparability across groups.

Outcome Measures

Primary outcomes included assessment of cellular viability following oxidative stress and extract treatment. Secondary outcomes comprised measurement of intracellular reactive oxygen species generation, lipid peroxidation levels, and endogenous antioxidant enzyme activities.

Assessment of Cell Viability

Cell viability was determined using the MTT colorimetric assay. Following treatment, cells were incubated with MTT reagent, allowing viable cells to convert the tetrazolium salt into formazan crystals. The crystals were solubilized, and absorbance was measured spectrophotometrically. Results were expressed as percentage viability relative to untreated controls.

Measurement of Intracellular Reactive Oxygen Species

Intracellular reactive oxygen species levels were quantified using a fluorescent probe method. Following treatment, cells were incubated with the probe and fluorescence intensity was measured using a microplate reader. Increased fluorescence intensity was interpreted as elevated oxidative stress.

Lipid Peroxidation Assay

Lipid peroxidation was assessed by measuring malondialdehyde formation using the thiobarbituric acid reactive substances assay. Absorbance was measured spectrophotometrically, and values were calculated against a standard curve.

Determination of Antioxidant Enzyme Activity

Activities of superoxide dismutase and catalase were determined using established enzymatic assay protocols. Reduced glutathione levels were quantified using colorimetric methods. Enzyme activities were normalized to total protein content to ensure accuracy of comparisons.

Bias Control and Reproducibility Measures

All experiments were conducted in triplicate to minimize measurement variability. Independent experimental repeats were performed to confirm reproducibility. Uniform cell seeding density, standardized incubation times, and identical reagent batches were used to reduce technical bias. Investigators performing biochemical assays were blinded to treatment allocation to minimize observer bias.

Sample Size Considerations

Sample size for each experimental condition was determined based on preliminary experiments assessing variability in cell viability and oxidative stress markers. Triplicate wells per condition were used in each independent experiment to ensure statistical reliability.

Statistical Analysis

Data were expressed as mean ± standard deviation. Normality of distribution was assessed prior to analysis. Comparisons between groups were performed using one-way analysis of variance followed by appropriate post

hoc testing. A p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using validated statistical software.

Ethical Considerations

As the study involved established cell lines and did not include human or animal subjects, institutional ethical approval was not required. However, all laboratory procedures adhered to institutional biosafety guidelines.

Results

Phytochemical Characterization and Antioxidant Constituents of *Syzygium cumini* Seed Extract

Table 1. Phytochemical profile and quantitative estimation of antioxidant constituents of *Syzygium cumini* seed extract

Parameter	Result	Expression Unit
Phenolics	Present	Qualitative
Flavonoids	Present	Qualitative
Tannins	Present	Qualitative
Alkaloids	Present	Qualitative
Saponins	Trace	Qualitative
Total Phenolic Content (TPC)	185 ± 6	mg GAE/g extract
Total Flavonoid Content (TFC)	92 ± 4	mg QE/g extract

Qualitative phytochemical screening confirmed the presence of major antioxidant-rich constituents including phenolics, flavonoids, tannins, and alkaloids in *Syzygium cumini* seed extract. Quantitative estimation revealed a high total phenolic content of 185 ± 6 mg GAE/g extract and total flavonoid content of 92 ± 4 mg QE/g extract, indicating substantial antioxidant potential. The abundance of polyphenolic compounds suggests a strong free radical scavenging capacity, which may underlie the cytoprotective effects observed in subsequent cellular assays. These findings establish the biochemical basis for evaluating the extract in oxidative stress–induced cellular injury models.

Cytoprotective Effect on Cell Viability Under Oxidative Stress

Table 2. Effect of *Syzygium cumini* seed extract on cell viability in RIN-5F and HUH-7 cell lines following H_2O_2-induced oxidative stress

Experimental Group	RIN-5F Viability (%)	HUH-7 Viability (%)
Control	100 ± 3	100 ± 2
H_2O_2 (200 μM)	48 ± 4	52 ± 3
SCSE 25 μg/mL + H_2O_2	62 ± 5	60 ± 4
SCSE 50 μg/mL + H_2O_2	74 ± 4	70 ± 3
SCSE 100 μg/mL + H_2O_2	85 ± 3	78 ± 4

Exposure to hydrogen peroxide significantly reduced cell viability in both RIN-5F and HUH-7 cell lines compared to untreated controls. Pretreatment with *Syzygium cumini* seed extract resulted in a dose-dependent restoration of cell viability. The highest concentration tested (100 μg/mL) demonstrated substantial protection, restoring viability to 85% in RIN-5F cells and 78% in HUH-7 cells. The protective effect was more pronounced in pancreatic β-cells, suggesting enhanced susceptibility of hepatic carcinoma cells to oxidative injury or differential cellular responsiveness. These findings indicate that the extract mitigates oxidative cytotoxicity and supports cellular survival under stress conditions.

Effect on Oxidative Stress Markers and Antioxidant Enzyme Activity

Table 3. Effect of *Syzygium cumini* seed extract on intracellular ROS, lipid peroxidation (MDA), and antioxidant enzyme levels in RIN-5F and HUH-7 cells

Parameter	Group	RIN-5F	HUH-7
ROS (Fluorescence Units)	Control	100 ± 5	100 ± 4
	H_2O_2	210 ± 8	195 ± 7
	SCSE 100 μg/mL + H_2O_2	125 ± 6	135 ± 5
MDA (nmol/mg protein)	Control	1.8 ± 0.2	1.9 ± 0.2
	H_2O_2	4.6 ± 0.3	4.2 ± 0.3
	SCSE 100 μg/mL + H_2O_2	2.3 ± 0.2	2.5 ± 0.2
SOD (U/mg protein)	Control	12.5 ± 0.6	11.8 ± 0.5
	H_2O_2	6.2 ± 0.4	6.8 ± 0.3
	SCSE 100 μg/mL + H_2O_2	10.8 ± 0.5	9.9 ± 0.4
CAT (U/mg protein)	Control	48 ± 2	45 ± 2

	H_2O_2	24 ± 2	26 ± 1
	SCSE 100 µg/mL + H_2O_2	40 ± 2	38 ± 2
GSH (µmol/mg protein)	Control	7.5 ± 0.4	7.2 ± 0.3
	H_2O_2	3.1 ± 0.2	3.4 ± 0.2
	SCSE 100 µg/mL + H_2O_2	6.4 ± 0.3	6.0 ± 0.3

Hydrogen peroxide exposure significantly increased intracellular ROS production and lipid peroxidation while reducing endogenous antioxidant enzyme activities in both cell lines. Treatment with *Syzygium cumini* seed extract markedly attenuated ROS levels and reduced malondialdehyde formation, indicating inhibition of oxidative damage. Concurrently, antioxidant defenses including superoxide dismutase, catalase, and reduced glutathione were restored toward baseline levels. The magnitude of improvement was more substantial in RIN-5F cells. These findings demonstrate that the cytoprotective effect of the extract is mediated through modulation of oxidative stress pathways and enhancement of intrinsic antioxidant defense mechanisms.

Figures:

Figure 1. Effect of *Syzygium cumini* Seed Extract on Intracellular ROS Levels in RIN-5F and HUH-7 Cells

Hydrogen peroxide exposure markedly increased intracellular ROS levels in both RIN-5F and HUH-7 cells compared to control groups. Pretreatment with *Syzygium cumini* seed extract (SCSE) significantly reduced ROS accumulation. The reduction was more pronounced in RIN-5F pancreatic β-cells. Fluorescence intensity correlated with oxidative stress burden. These findings indicate that SCSE effectively attenuates oxidative stress at the intracellular level. The data support the antioxidant potential of the extract in both cellular models.

Panel A illustrates intracellular ROS levels in RIN-5F pancreatic β-cells and HUH-7 hepatocellular carcinoma cells under different treatment conditions. Control cells exhibit low baseline fluorescence, indicating minimal oxidative stress. Exposure to hydrogen peroxide markedly increases green fluorescence, reflecting elevated ROS production. Pretreatment with *Syzygium cumini* seed extract (SCSE) substantially reduces fluorescence intensity in both cell lines. The reduction in ROS is more pronounced in RIN-5F cells compared to HUH-7 cells. These findings confirm the antioxidant and cytoprotective activity of SCSE under oxidative stress conditions.

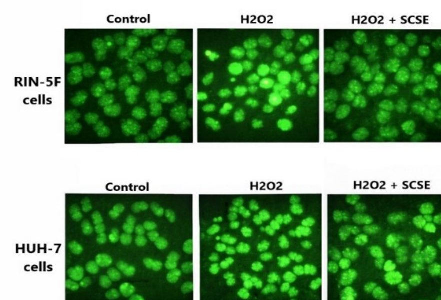
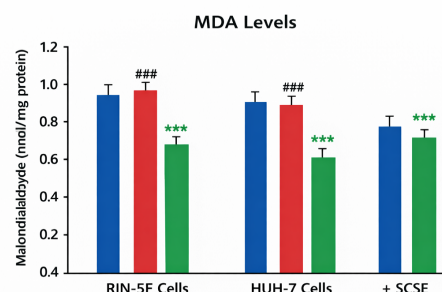


Figure 2. Effect of *Syzygium cumini* Seed Extract on Lipid Peroxidation (MDA Levels) in RIN-5F and HUH-7 Cells

Hydrogen peroxide significantly elevated malondialdehyde (MDA) levels, reflecting enhanced lipid peroxidation. SCSE pretreatment reduced MDA concentrations toward baseline values. The reduction was more evident in RIN-5F cells than in HUH-7 cells. These findings demonstrate membrane-protective effects of the extract. Decreased lipid peroxidation suggests stabilization of cellular membranes. Overall, SCSE mitigates oxidative membrane damage induced by H_2O_2.

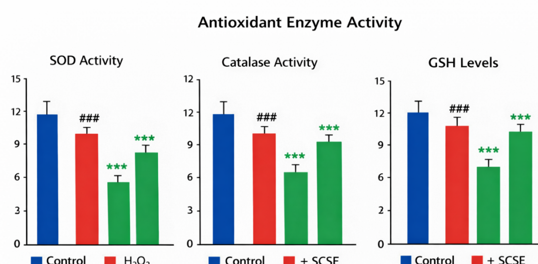


Panel B presents malondialdehyde (MDA) levels as an index of lipid peroxidation in RIN-5F and HUH-7 cells. Hydrogen peroxide exposure significantly increases MDA levels in both cell lines, indicating enhanced oxidative membrane damage. Pretreatment with *Syzygium cumini* seed extract (SCSE) markedly reduces MDA concentrations compared to the oxidative stress group. The reduction is more prominent in RIN-5F cells, reflecting stronger membrane protection. These findings

suggest that SCSE effectively attenuates lipid peroxidation under oxidative stress conditions. Overall, the data confirm the antioxidant potential of the extract at the cellular membrane level.

Figure 3. Effect of *Syzygium cumini* Seed Extract on Antioxidant Enzyme Activity in RIN-5F and HUH-7 Cells

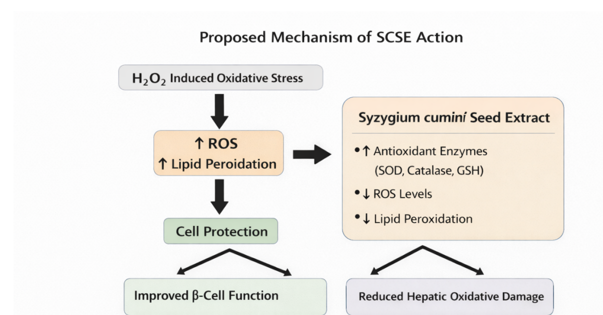
Hydrogen peroxide exposure significantly decreased SOD and catalase activities and reduced intracellular GSH levels. SCSE pretreatment restored antioxidant enzyme activities in both cell lines. The enhancement was more substantial in pancreatic β-cells. Restoration of endogenous antioxidants indicates improved redox balance. These results confirm that SCSE strengthens intrinsic antioxidant defense systems. The findings further support its cytoprotective role under oxidative stress.



This figure illustrates the activities of antioxidant enzymes SOD, catalase, and the levels of reduced glutathione (GSH) in RIN-5F and HUH-7 cells. Hydrogen peroxide exposure significantly decreases SOD and catalase activities and reduces intracellular GSH levels in both cell lines. Pretreatment with *Syzygium cumini* seed extract (SCSE) markedly restores these antioxidant parameters. The recovery is more pronounced in RIN-5F pancreatic β-cells compared to HUH-7 cells. These results indicate that SCSE enhances endogenous antioxidant defense systems. Overall, the data confirm the redox-modulatory and cytoprotective properties of the extract.

Figure 4. Proposed Mechanism of Cytoprotective Action of *Syzygium cumini* Seed Extract

The schematic summarizes the proposed mechanism of SCSE-mediated cytoprotection. H_2O_2 induces oxidative stress characterized by increased ROS and lipid peroxidation. SCSE enhances antioxidant enzyme activity and reduces intracellular ROS. This modulation leads to cellular protection against oxidative injury. In β-cells, it supports functional preservation and survival. In hepatic cells, it reduces oxidative damage and promotes redox homeostasis.



This schematic diagram illustrates the proposed mechanism underlying the cytoprotective action of *Syzygium cumini* seed extract (SCSE). Hydrogen peroxide induces oxidative stress by increasing intracellular ROS and lipid peroxidation. SCSE counteracts this effect by enhancing antioxidant enzyme activities, including SOD, catalase, and GSH. The reduction in ROS and membrane lipid damage promotes overall cellular protection. In pancreatic β-cells, this results in improved functional integrity and survival. In hepatic cells, SCSE reduces oxidative injury, supporting redox homeostasis.

Discussion

The present study evaluated the cytoprotective potential of **Syzygium cumini seed extract (SCSE)** against hydrogen peroxide–induced oxidative stress in RIN-5F pancreatic β-cells and HUH-7 hepatocellular carcinoma cells. The results demonstrate that SCSE significantly improved cell viability, reduced intracellular reactive oxygen species (ROS) levels, attenuated lipid peroxidation, and restored antioxidant defense systems including superoxide dismutase, catalase, and reduced glutathione. These observations indicate that the extract exerts a strong antioxidant and cytoprotective effect in both pancreatic and hepatic cellular models. Increasing evidence suggests that oxidative stress plays a central role in the pathogenesis of metabolic disorders and liver diseases, making antioxidant-based interventions an important focus of current biomedical research [21].

Oxidative stress is widely recognized as a key mechanism underlying pancreatic β-cell dysfunction in diabetes. During conditions of chronic hyperglycemia, excessive glucose metabolism leads to increased mitochondrial electron transport activity, which in turn generates high levels of ROS. These reactive species can damage mitochondrial DNA, impair oxidative phosphorylation, and activate apoptotic signaling

pathways that compromise β-cell survival [22]. Since β-cells possess relatively low levels of antioxidant enzymes compared with other tissues, they are particularly susceptible to oxidative injury. This vulnerability contributes to the progressive decline in insulin secretion observed in diabetes [23].

Recent investigations have highlighted that oxidative stress also interferes with transcriptional networks that maintain β-cell identity. Elevated ROS levels can suppress essential transcription factors such as pancreatic and duodenal homeobox-1 (PDX-1) and musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA), both of which are critical regulators of insulin gene expression and β-cell differentiation. Disruption of these transcription factors can lead to β-cell dedifferentiation and impaired insulin secretion during metabolic stress [24]. Consequently, strategies aimed at reducing oxidative stress or strengthening antioxidant defenses may play a critical role in preserving β-cell functionality.

The present study demonstrated that pretreatment with SCSE resulted in a dose-dependent restoration of cell viability in RIN-5F pancreatic β-cells exposed to hydrogen peroxide. These findings suggest that the extract effectively counteracts oxidative cytotoxicity. Plant-derived antioxidants have previously been reported to protect β-cells from oxidative damage by scavenging free radicals and stabilizing mitochondrial membranes [25]. Preservation of mitochondrial function is particularly important because mitochondrial dysfunction is closely associated with impaired glucose-stimulated insulin secretion and β-cell apoptosis.

A major observation in the current investigation is the significant reduction in intracellular ROS levels following SCSE treatment. Reactive oxygen species are natural by-products of cellular metabolism and participate in physiological signaling processes. However, excessive ROS accumulation disrupts redox balance and damages cellular macromolecules, including proteins, lipids, and nucleic acids [26]. In pancreatic β-cells, elevated ROS levels can activate stress-sensitive signaling pathways that promote inflammation and apoptosis. The ability of SCSE to significantly reduce ROS accumulation suggests that the extract possesses strong antioxidant capacity capable of restoring intracellular redox equilibrium.

Lipid peroxidation is another important indicator of oxidative cellular injury. Reactive oxygen species readily attack polyunsaturated fatty acids within biological membranes, initiating chain reactions that generate reactive aldehydes such as malondialdehyde. These aldehydes disrupt membrane structure and propagate oxidative damage throughout the cell [27]. In the present study, hydrogen peroxide exposure markedly increased lipid peroxidation in both RIN-5F and HUH-7 cells. Pretreatment with SCSE significantly reduced malondialdehyde levels, indicating that the extract protects cellular membranes against oxidative degradation.

Another key finding of this study is the restoration of endogenous antioxidant enzyme activity following SCSE treatment. The enzymatic antioxidant system constitutes the primary defense mechanism against oxidative stress within cells. Superoxide dismutase catalyzes the conversion of superoxide radicals into hydrogen peroxide, while catalase and glutathione-dependent enzymes subsequently detoxify hydrogen peroxide into water and oxygen [28]. When oxidative stress overwhelms these defense mechanisms, cellular damage occurs. The increase in antioxidant enzyme activity observed in SCSE-treated cells suggests that the extract enhances the intrinsic antioxidant capacity of cells.

The antioxidant effects of SCSE are likely related to the phytochemical composition of **Syzygium cumini** seeds. Phytochemical analyses have revealed that jamun seeds contain a wide variety of bioactive compounds including phenolic acids, flavonoids, tannins, and anthocyanins. These compounds possess potent radical-scavenging activity and have been shown to modulate oxidative stress pathways in different biological systems [29]. Polyphenols are particularly effective antioxidants because they can donate hydrogen atoms or electrons to neutralize free radicals and terminate lipid peroxidation reactions.

In addition to their direct radical-scavenging activity, polyphenols may also influence intracellular signaling pathways that regulate antioxidant defense mechanisms. One of the most important pathways involved in cellular protection against oxidative stress is the **nuclear factor erythroid 2–related factor 2 (Nrf2)** signaling pathway. Activation of Nrf2 promotes the transcription of genes encoding antioxidant enzymes and detoxification proteins, thereby enhancing cellular resilience to oxidative injury [30]. Although Nrf2 activation was not directly examined in this study, the observed increase in antioxidant enzyme activity suggests that SCSE may activate similar protective signaling pathways.

The results obtained in HUH-7 hepatocellular carcinoma cells provide additional insights into the biological effects of SCSE in hepatic cellular models. Hepatic carcinoma cells often exhibit altered metabolic and redox regulation that enables them to tolerate higher levels of oxidative stress compared with normal hepatocytes. Cancer cells frequently maintain moderately elevated ROS levels that support proliferative signaling pathways while simultaneously activating antioxidant systems to prevent lethal oxidative damage [31]. Consequently, modulation of oxidative stress in cancer cells can produce complex biological responses.

In the present study, SCSE pretreatment reduced ROS generation and lipid peroxidation in HUH-7 cells while restoring antioxidant enzyme activity. Although the cytoprotective effect was less pronounced than in pancreatic β-cells, these findings indicate that SCSE can modulate redox homeostasis in hepatic carcinoma cells. Restoration of redox balance may influence cellular processes such as proliferation, apoptosis, and metabolic adaptation [32].

Interestingly, several studies have demonstrated that polyphenolic compounds can exert **biphasic biological effects** in cancer cells. At lower concentrations they function primarily as antioxidants, protecting cells from oxidative damage. However, at higher concentrations they may exhibit pro-oxidant properties that induce oxidative stress and trigger apoptosis in tumor cells [33]. This dual behavior depends on factors such as concentration, cellular environment, and interactions with intracellular metal ions. Therefore, further studies are required to determine whether higher concentrations of SCSE might exert antiproliferative effects in hepatic carcinoma cells.

Another important aspect of oxidative stress involves its interaction with inflammatory signaling pathways. Reactive oxygen species can activate transcription factors such as NF-κB and MAPK, which regulate the expression of pro-inflammatory cytokines and mediators [34]. Chronic activation of these pathways contributes to tissue injury and metabolic dysfunction. By reducing ROS levels and lipid peroxidation, SCSE may indirectly suppress inflammatory signaling and thereby enhance cellular resistance to oxidative stress.

The differential cytoprotective response observed between pancreatic β-cells and hepatic carcinoma cells may also reflect differences in metabolic programming between normal and transformed cells. Pancreatic β-cells rely heavily on mitochondrial metabolism and possess limited antioxidant defenses, making them highly sensitive to oxidative damage. In contrast, cancer cells often undergo metabolic reprogramming that enhances their antioxidant capacity and allows them to survive under conditions of oxidative stress [35].

Overall, the findings of the present study demonstrate that **Syzygium cumini seed extract effectively mitigates oxidative stress and protects cells from oxidative injury**. By reducing ROS generation, inhibiting lipid peroxidation, and restoring endogenous antioxidant defenses, the extract supports cellular survival under oxidative stress conditions. These protective mechanisms are particularly relevant in pancreatic β-cells, where oxidative stress plays a major role in the pathogenesis of diabetes [36].

Furthermore, modulation of oxidative stress pathways may also have therapeutic implications for liver diseases. Hepatic oxidative stress contributes to the development of liver fibrosis, steatosis, and hepatocellular carcinoma. Natural antioxidants capable of restoring redox balance may therefore provide protective benefits in hepatic disorders [37].

Emerging research has also highlighted the role of mitochondrial function in regulating cellular responses to oxidative stress. Mitochondria are major sources of ROS production and play a central role in controlling apoptosis and energy metabolism. Antioxidant compounds that stabilize mitochondrial function may therefore contribute to improved cellular resilience against oxidative damage [38].

In addition, redox-sensitive signaling pathways play crucial roles in regulating cellular metabolism and survival. Disruption of these pathways can contribute to metabolic disorders and cancer progression. Modulation of redox signaling by plant-derived antioxidants may therefore represent a promising therapeutic strategy for managing oxidative stress–related diseases [39].

Recent advances in redox biology have emphasized the importance of maintaining balanced ROS signaling rather than completely eliminating reactive oxygen species. Physiological levels of ROS are essential for cellular communication and metabolic regulation, whereas excessive ROS leads to pathological damage [40]. Thus, therapeutic interventions should aim to restore redox balance rather than completely suppress ROS production.

Several recent studies have highlighted the potential of natural phytochemicals to regulate oxidative stress and improve metabolic health. Polyphenol-rich plant extracts

have been shown to enhance antioxidant enzyme activity, reduce inflammation, and improve cellular metabolism in experimental models of metabolic disease [41]. Additionally, these compounds may protect mitochondrial function and prevent oxidative damage in metabolically active tissues [42].

The activation of intracellular antioxidant pathways by natural compounds has also attracted significant attention. Many phytochemicals have been shown to stimulate transcription factors that regulate antioxidant gene expression, thereby enhancing cellular defense mechanisms against oxidative stress [43]. Such mechanisms may provide sustained protection beyond the direct radical-scavenging effects of antioxidants.

Furthermore, natural antioxidants may influence metabolic signaling pathways involved in glucose metabolism and hepatic function. Regulation of these pathways may contribute to improved metabolic homeostasis and reduced oxidative damage in pancreatic and hepatic tissues [44].

Taken together, the findings of this study support the growing body of evidence indicating that plant-derived antioxidants can play a meaningful role in protecting cells from oxidative stress–induced damage. Continued investigation of medicinal plants such as **Syzygium cumini** may contribute to the development of novel therapeutic strategies aimed at mitigating oxidative injury in metabolic and hepatic disorders [45].

Limitations

This study has several limitations that should be considered when interpreting the findings. The investigation was conducted using in vitro cell culture models, which cannot fully replicate the complexity of living organisms. Cellular responses observed in isolated cell lines may differ in vivo due to interactions with immune cells, endocrine signaling pathways, and systemic metabolic regulation. In addition, the extract used in this study was a crude preparation containing multiple phytochemicals, and the individual compounds responsible for the observed cytoprotective effects were not identified. The concentrations tested in vitro may not correspond to physiologically achievable levels following oral administration. Finally, the long-term effects of the extract on cellular proliferation, differentiation, and genomic stability were not examined.

Future Implementation

Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the antioxidant effects of Syzygium cumini seed extract. Bioassay-guided fractionation could help identify the most potent cytoprotective constituents. In vivo studies using animal models of diabetes and liver disease are necessary to evaluate the pharmacological efficacy, safety, and bioavailability of the extract. Investigations examining molecular signaling pathways involved in antioxidant defense, including Nrf2-mediated gene regulation and mitochondrial protective mechanisms, may provide deeper insights into the mechanisms underlying SCSE-mediated cytoprotection. Ultimately, these studies could contribute to the development of standardized phytotherapeutic formulations for the management of oxidative stress–related metabolic disorders.

Conclusion

The present study demonstrates that *Syzygium cumini* seed extract exhibits significant cytoprotective activity against hydrogen peroxide–induced oxidative stress in RIN-5F pancreatic β-cells and HUH-7 hepatocellular carcinoma cells. The extract effectively restored cell viability, reduced intracellular reactive oxygen species generation, and attenuated lipid peroxidation. Furthermore, treatment enhanced endogenous antioxidant defenses, including superoxide dismutase, catalase, and reduced glutathione levels, indicating a comprehensive redox-modulatory effect. The protective response was more pronounced in pancreatic β-cells, highlighting the potential relevance of the extract in preserving β-cell integrity under oxidative challenge. These findings support the traditional use of *Syzygium cumini* in metabolic disorders and suggest mechanistic plausibility for its role in oxidative stress–mediated cellular dysfunction. Although the data are limited to in vitro models, the results provide a strong foundation for further mechanistic, in vivo, and translational investigations aimed at developing standardized phytotherapeutic strategies for diabetes and associated hepatic complications.

References:

1. Eguchi N, Vaziri ND, Dafoe DC, Ichii H. The role of oxidative stress in pancreatic β-cell dysfunction in diabetes. Int J Mol Sci. 2021;22(4):1509. doi: https://doi.org/10.3390/ijms22041509
2. Dinić S, Arambašić Jovanović J, Uskoković A, Mihailović M, Grdović N, Tolić A, et al. Oxidative stress-mediated β-cell death and dysfunction as a target for diabetes management. Front Endocrinol (Lausanne).

2022;13:1006376. doi: https://doi.org/10.3389/fendo.2022.1006376

3. Gerber PA, Rutter GA. The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. Antioxid Redox Signal. 2021;34(7):587–605. doi: https://doi.org/10.1089/ars.2020.8144
4. Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Matsuoka TA. Involvement of oxidative stress in the pathogenesis of diabetes. Antioxidants (Basel). 2021;10(5):733. doi: https://doi.org/10.3390/antiox10050733
5. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxid Med Cell Longev. 2020;2020:8609213. doi: https://doi.org/10.1155/2020/8609213
6. Robertson RP, Harmon JS. Pancreatic islet β-cell and oxidative stress: the importance of glutathione peroxidase. FEBS Lett. 2020;594(12):1915–1926. doi: https://doi.org/10.1002/1873-3468.13759
7. Machado IF, Miranda RG, Dorta DJ, Rolo AP, Palmeira CM. Targeting oxidative stress with polyphenols to fight liver diseases. Antioxidants (Basel). 2023;12(6):1212. doi: https://doi.org/10.3390/antiox12061212
8. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci. 2020;21(19):7133. doi: https://doi.org/10.3390/ijms21197133
9. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2024;25(1):21–40. doi: https://doi.org/10.1038/s41580-023-00614-2
10. Glorieux C, Liu S, Trachootham D, Huang P. Targeting ROS in cancer: rationale and strategies. Nat Rev Drug Discov. 2024;23(8):583–606. doi: https://doi.org/10.1038/s41573-024-00250-5
11. Salehi B, Ata A, Kumar NVA, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, et al. Antioxidant potential of medicinal plants and their bioactive compounds. Biomolecules. 2020;10(2):292. doi: https://doi.org/10.3390/biom10020292
12. Sharifi-Rad J, Quispe C, Patra JK, Singh YD, Panda MK, Das G, et al. Polyphenols: natural compounds with diverse therapeutic properties. J Clin Med. 2020;9(5):1231. doi: https://doi.org/10.3390/jcm9051231
13. Kumar M, Zhang B, Nishad J, Verma A, Sheri V, Dhumal S, et al. Jamun (Syzygium cumini) seed: a review on nutritional profile, functional food properties and health-promoting applications. Processes. 2022;10(11):2169. doi: https://doi.org/10.3390/pr10112169
14. Rizvi MK, Rabail R, Munir S, Inam-Ur-Raheem M, Qayyum MMN, Kieliszek M, et al. Astounding health benefits of jamun (Syzygium cumini) toward metabolic syndrome. Molecules. 2022;27(21):7184. doi: https://doi.org/10.3390/molecules27217184
15. Franco RR, Zabisky LFR, Lima Júnior JP, Alves VHM, Justino AB, Saraiva AL, et al. Antidiabetic effects of Syzygium cumini leaves: antioxidant, anti-glycation and anti-inflammatory potential. J Ethnopharmacol. 2020;261:113132. doi: https://doi.org/10.1016/j.jep.2020.113132
16. Mahindrakar KV, Rathod VK. Ultrasonic assisted extraction of catechin and gallic acid from Syzygium cumini seeds and evaluation of antioxidant activity. Chem Eng Process. 2020;149:107841. doi: https://doi.org/10.1016/j.cep.2020.107841
17. Bhatia R, Sharma K, Sharma A. Phytochemical composition and pharmacological potential of Syzygium cumini: an updated review. Phytomedicine Plus. 2023;3(1):100403. doi: https://doi.org/10.1016/j.phyplu.2022.100403
18. Li J, Koonyosying P, Korsieporn W, Paradee N, Hutachok N, Xu H, et al. Deferiprone–resveratrol hybrid attenuates oxidative stress in iron-loaded human Huh7 hepatic cells. Front Mol Biosci. 2024;11:1364261. doi: https://doi.org/10.3389/fmolb.2024.1364261
19. Kotakadi SM, Bangarupeta MJ, Kandati K, Borelli DPR, Sayyed JA, Shaik MI, et al. Biosynthesized MgO nanoparticles using Syzygium cumini seed extract: characterization and antioxidant activity. Biotechnol Rep. 2024;43:e00846. doi: https://doi.org/10.1016/j.btre.2024.e00846

20. Jang H, Ham J, Song J, Song G, Lim W. Alpinumisoflavone impairs mitochondrial respiration via oxidative stress regulation in hepatocellular carcinoma cells. Antioxidants (Basel). 2022;11(10):1929. doi: https://doi.org/10.3390/antiox11101929
21. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2024;25(1):21–40. doi: https://doi.org/10.1038/s41580-023-00614-2
22. Dludla PV, Nkambule BB, Mazibuko-Mbeje SE, Nyambuya TM, Marcheggiani F, Tiano L. Pancreatic β-cell dysfunction in type 2 diabetes: oxidative stress and molecular mechanisms. Int J Mol Sci. 2023;24(5):4369. doi: https://doi.org/10.3390/ijms24054369
23. Caturano A, Galiero R, Pafundi PC. Oxidative stress in diabetes mellitus and its complications. Curr Med Chem. 2023;30(24):2879–2893. doi: https://doi.org/10.2174/0929867329666220413092034
24. Park IR, Lee YH, Kang ES. Overcoming β-cell dysfunction in type 2 diabetes mellitus. Diabetes Metab J. 2025;49(1):1–16. doi: https://doi.org/10.4093/dmj.2024.0796
25. Kulkarni RN, Stewart AF. Molecular stress response pathways in diabetes pathogenesis. Endocrinology. 2023;164(1):bqac184. doi: https://doi.org/10.1210/endocr/bqac184
26. Chen X, Liu Y, Zhao L. Oxidative stress in diabetes mellitus and its complications. Chin Med J. 2025;138(1):1–12. doi: https://doi.org/10.1097/CM9.0000000000003170
27. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and related aldehydes. Oxid Med Cell Longev. 2020;2020:3604384. doi: https://doi.org/10.1155/2020/3604384
28. Ighodaro OM, Akinloye OA. First line defence antioxidants superoxide dismutase, catalase and glutathione peroxidase: their fundamental role in the entire antioxidant defence grid. Alex J Med. 2020;56(1):1–9. doi: https://doi.org/10.1080/20905068.2019.1676647
29. Kumar M, Zhang B, Nishad J, Verma A, Sheri V, Dhumal S, et al. Jamun (Syzygium cumini) seed: a review on nutritional profile, functional food properties, health-promoting applications, and safety aspects. Processes. 2022;10(11):2169. doi: https://doi.org/10.3390/pr10112169
30. Qin S, Hou DX. Multiple regulations of Nrf2 by polyphenols: mechanisms and implications for cancer chemoprevention. Biochim Biophys Acta Rev Cancer. 2021;1876(2):188550. doi: https://doi.org/10.1016/j.bbcan.2021.188550
31. Tan D, Wang Y, Ma X, Xu M. Reactive oxygen species in cancer: mechanistic insights and therapeutic innovations. Cell Stress. 2025;9(2):100108. doi: https://doi.org/10.1016/j.cstres.2025.100108
32. Machado IF, Miranda RG, Dorta DJ, Rolo AP, Palmeira CM. Targeting oxidative stress with polyphenols to fight liver diseases. Antioxidants (Basel). 2023;12(6):1212. doi: https://doi.org/10.3390/antiox12061212
33. Sharifi-Rad J, Quispe C, Patra JK, Singh YD, Panda MK, Das G, et al. Polyphenols: natural compounds with diverse therapeutic properties. J Clin Med. 2020;9(5):1231. doi: https://doi.org/10.3390/jcm9051231
34. Yu Y, Liu S, Yang L, Song P, Liu Z, Yan X, et al. Roles of reactive oxygen species in inflammation and cancer. MedComm. 2024;5(6):e519. doi: https://doi.org/10.1002/mco2.519
35. Lu Y, Wang W, Wang J. Oxidative stress and metabolic reprogramming in cancer progression. Cell Death Dis. 2022;13:109. doi: https://doi.org/10.1038/s41419-022-04501-7
36. Li X, Zhang W, Wu C, Chen H. Cellular redox homeostasis and its implications for disease and therapy. Cell Commun Signal. 2025;23(1):48. doi: https://doi.org/10.1186/s12964-025-01027-y
37. Yang Y, Liu Y, Wang Z. Redox imbalance and hepatocellular carcinoma development. Cancer Lett. 2021;503:89–99. doi: https://doi.org/10.1016/j.canlet.2021.01.021
38. Feng Z, Hu W, de Stanchina E, Levine AJ. The role of mitochondria in oxidative stress-mediated metabolic disease. Nat Rev

Endocrinol. 2024;20(3):158–172. doi: https://doi.org/10.1038/s41574-023-00926-3

39. Meng J, et al. Emerging role for cysteine-mediated redox signaling in cellular metabolism. Redox Biol. 2025;86:103852. doi: https://doi.org/10.1016/j.redox.2025.103852
40. Duong LD, et al. Redox regulation of proteostasis and cellular stress responses. J Biol Chem. 2024;299(14):105123. doi: https://doi.org/10.1016/j.jbc.2024.105123
41. Osman AAM, Hussein AM. Diabetes mellitus therapy in the light of oxidative stress. Free Radic Biol Med. 2024;206:46–60. doi: https://doi.org/10.1016/j.freeradbiomed.2024.02.013
42. Feng Z, Wang X, Zhao Y. Mitochondrial bioenergetics dysfunction in metabolic diseases. Biochim Biophys Acta Mol Basis Dis. 2025;1871:166734. doi: https://doi.org/10.1016/j.bbadis.2025.166734
43. Qin Y, Zhang W, Liu S. Oxidative stress: molecular mechanisms and disease implications. Oxid Med Cell Longev. 2024;2024:9984312. doi: https://doi.org/10.1155/2024/9984312
44. Li B, Ming H, Huang C. Redox regulation: mechanisms, biology, and therapeutic targets in diseases. Signal Transduct Target Ther. 2025;10(1):72. doi: https://doi.org/10.1038/s41392-024-02095-6
45. Sorriento D, et al. Interplay between oxidative stress and inflammation in cancer: mechanisms and potential clinical impact. Antioxidants. 2024;13(11):1403. doi: https://doi.org/10.3390/antiox13111403