



Review Article

Theaflavin-3,3' -Digallate Encapsulated β -Chitosan Nanoparticles for the Prevention of Chemotherapy-Induced Toxicity: Mechanisms, Therapeutic Potential, and Future Perspectives

Taniya Mary Martin, Meenakshi Sundaram Kishore Kumar*

Department of Anatomy, Zebrafish Facility, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 600077, Tamil Nadu, India

Received: 5 June 2026

Accepted: 27 June 2026

Published online: 30 June 2026

Keywords: cancer, human, health, green products, nanotechnology

Chemotherapy remains a cornerstone of cancer management; however, its clinical effectiveness is often compromised by severe systemic toxicity that limits therapeutic dosing and adversely affects patient quality of life. In recent years, natural polyphenols have gained attention as adjunctive agents capable of mitigating chemotherapy induced adverse effects. Among these, theaflavin-3',3'-digallate (TFDG), a major bioactive constituent of black tea, exhibits potent antioxidant, anti-inflammatory, and antitumour activities. Despite its therapeutic promise, the clinical translation of TFDG is hindered by poor aqueous solubility, low bioavailability, and rapid metabolic degradation. Nanotechnology based delivery systems, particularly biopolymeric carriers such as β -chitosan nanoparticles (β -CS-NPs), offer a viable strategy to overcome these limitations. Chitosan based nanoparticles provide enhanced stability, controlled release, improved cellular uptake, and favourable biocompatibility, making them suitable vehicles for polyphenol delivery. This review critically summarizes current knowledge on the chemical characteristics and biological functions of TFDG, recent advances in β -chitosan nanoparticle formulations, and their combined potential to act as a biological shield against chemotherapy-induced toxicity. The integration of TFDG with β -CS-NPs represents a promising nanotherapeutic approach to improve treatment tolerance with preserving anticancer efficacy.

Introduction

Chemotherapy remains one of the most widely employed therapeutic strategies for the management of both solid and haematological malignancies [1]. Despite substantial progress in targeted therapies and immunotherapy, conventional chemotherapeutic agents such as cisplatin, doxorubicin, cyclophosphamide, and 5-fluorouracil continue to play a central role in cancer treatment protocols. However, the clinical utility of chemotherapy is significantly constrained by its lack of selectivity, leading to severe systemic toxicity in rapidly dividing normal tissues. Chemotherapy induced adverse effect, including oxidative stress, inflammation, organ dysfunction, immunosuppression, and gastrointestinal toxicity, often result in dose reduction or treatment discontinuation, thereby compromising therapeutic outcomes and patient quality

of life [2].

A key mechanism underlying chemotherapy associated with toxicity is the excessive generation of reactive oxygen species (ROS), which disrupt cellular redox homeostasis and damage critical biomolecules such as lipids, proteins, and nucleic acids [3]. Elevated oxidative stress activates inflammatory cascades, mitochondrial dysfunction, DNA damage responses, and apoptotic signaling in healthy tissues, particularly in the liver, kidneys, heart, and gastrointestinal tract. While cancer cells are often more susceptible to oxidative damage due to their altered metabolism, normal tissues lack sufficient protective mechanisms to withstand prolonged chemotherapeutic insult. Therefore, the development of adjunctive strategies that selectively protect normal cells without attenuating anticancer efficacy has emerged as a crucial research priority [4].

*Corresponding author: Dr. Meenakshi Sundaram Kishore Kumar
 E-mail address: meenakshisundaram.sdc@saveetha.com

<https://doi.org/10.65795/t8rm8k88>

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In this context, naturally derived bioactive compounds have gained increasing attention as potential chemoprotective agents. Polyphenols, a diverse class of plant derived secondary metabolites, exhibit strong antioxidant, anti-inflammatory, and cytoprotective properties. Epidemiological and experimental studies suggest that dietary polyphenols may modulate cancer risk and improve tolerance to anticancer therapies [5]. Among these compounds, tea polyphenols have been extensively investigated due to their broad biological activities and favorable safety profiles. While green tea catechins such as epigallocatechin gallate (EGCG) have been widely studied, black tea derived theaflavins have recently emerged as potent bioactive molecules with distinct pharmacological properties [6].

Theaflavin-3',3'-digallate (TFDG) is a major theaflavin formed during the enzymatic oxidation of catechins in black tea processing. Structurally, TFDG possesses multiple hydroxyl groups and gallate moieties, conferring strong free radical scavenging capacity and metal chelation ability [7]. Accumulating evidence indicates that TFDG exerts a wide range of biological effects, including antioxidant, anti-inflammatory, antiproliferative, and pro-apoptotic activities in various cancer cell models. Importantly, TFDG has been shown to modulate key signalling pathways involved in oxidative stress response, mitochondrial function, and cell survival, suggesting its potential role as a protective agent against chemotherapy induced cellular damage [8].

Despite these promising attributes, the clinical application of TFDG remains limited by several pharmacokinetic challenges. Like many polyphenols, TFDG exhibits poor aqueous solubility, low intestinal permeability, rapid metabolic degradation, and limited systemic bioavailability. Following oral administration, TFDG undergoes extensive first pass metabolism, resulting in low plasma concentrations that are often insufficient to elicit therapeutic effects in target tissues. These limitations significantly hinder the translation of TFDG from *in vitro* studies to *in vivo* and clinical settings. Consequently, innovative delivery strategies are required to improve the stability, bioavailability, and therapeutic efficacy of TFDG [9].

Nanotechnology based drug delivery systems have emerged as powerful tools to overcome the pharmacokinetic and biological limitations of bioactive compounds [10]. Nanocarriers can protect encapsulated agents from premature degradation, enhance solubility, improve cellular uptake, and enable controlled or targeted release. Among the various nanomaterials explored, biopolymeric nanoparticles have attracted particular interest due to their biocompatibility, biodegradability, and low toxicity. Chitosan, a natural polysaccharide derived from chitin, has emerged as a versatile and widely studied biopolymer for drug delivery applications [11].

Chitosan is composed of β -(1 $\rightarrow$ 4)-linked D-glucosamine and N-acetyl-D-glucosamine units and is characterized by its positive surface charge, mucoadhesive properties, and ability to form nanoparticles through simple ionic gelation techniques [12]. These features enable chitosan nanoparticles to interact effectively with negatively charged biological membranes, thereby enhancing cellular internalization and trans-epithelial transport. Moreover, β -chitosan exhibits intrinsic antioxidant,

antimicrobial, and immunomodulatory properties, further supporting its suitability as a therapeutic carrier [13].

The present review specifically focuses on β -chitosan, a less extensively explored but highly promising chitosan allomorph obtained primarily from squid pens. Unlike the conventional α -chitosan, which possesses an antiparallel crystalline arrangement stabilized by strong inter-molecular hydrogen bonding, β -chitosan exhibits a parallel-chain structure with comparatively weaker hydrogen-bond interactions [14]. This structural distinction results in greater chain flexibility, higher hydration capacity, and increased swelling behavior, thereby facilitating improved interaction with aqueous biological environments. In addition, the more open crystalline organization of β -chitosan enhances accessibility of amino groups generated through deacetylation, promoting more efficient ionic crosslinking, drug encapsulation, and nanoparticle formation. The higher swelling ratio and enhanced solubility of β -chitosan further contribute to superior drug loading, controlled release characteristics, and biodegradation kinetics compared with β -chitosan [15]. These physicochemical advantages make β -chitosan a particularly attractive carrier for polyphenolic compounds such as TFDG, justifying its selection as the biomaterial platform highlighted in this review.

Encapsulation of polyphenols within β -chitosan nanoparticles has been shown to significantly improve their stability, bioavailability, and biological activity. β -Chitosan based delivery systems can protect polyphenols from harsh gastrointestinal conditions, reduce enzymatic degradation, and facilitate sustained release at target sites. Importantly, β -chitosan nanoparticles can be engineered to respond to pH changes or oxidative environments, enabling selective release in inflamed or damaged tissues commonly associated with chemotherapy induced toxicity. Such properties position β -chitosan nanoparticles as promising vehicles for the delivery of chemoprotective agents [16].

The combination of TFDG with β -chitosan nanoparticles (TFDG- β -CS-NPs) represents a novel and rational strategy to harness the therapeutic potential of black tea polyphenols while addressing their inherent limitations. By enhancing the bioavailability and stability of TFDG, β -chitosan nanoparticles may amplify its antioxidant and cytoprotective effects, thereby acting as a biological shield against chemotherapy induced oxidative stress and organ damage [17]. Additionally, emerging evidence suggests that nanoparticle mediated delivery may allow for selective protection of normal cells while preserving, or even enhancing, the anticancer activity of chemotherapeutic agents.

This review aims to provide a comprehensive overview of the current understanding of TFDG and β -chitosan nanoparticle systems in the context of chemotherapy toxicity. It synthesizes recent advances in the chemistry, biological activities, and pharmacological challenges of TFDG, highlights the design and functional attributes of β -chitosan nanoparticles, and discusses the mechanistic rationale for their combined use as chemoprotective nanotherapeutics. By integrating insights from cancer biology, natural product research, and nanomedicine, this review seeks to elucidate the potential of TFDG- β -CS-NPs as an innovative strategy to improve chemotherapy tolerance and therapeutic outcomes.

Table 1: Biological Activities and Limitations of TFDG

Category	Key Findings	Mechanism/Notes	Limitations
Antioxidant Activity	Scavenges ROS, reduces lipid peroxidation	Free radical neutralization, metal chelation	Rapid degradation under physiological conditions [22].
Anticancer Activity	Inhibits proliferation, enhances chemosensitivity	Modulates glutathione, copper transporter pathways, induces apoptosis	Poor intestinal permeability, low systemic bioavailability [23].
Cytoprotective Effects	Protects normal cells from oxidative stress	Activates Nrf2/GPX4, inhibits ferroptosis	Unstable in GI tract; rapid metabolism [24].
Anti-inflammatory Activity	Reduces pro-inflammatory cytokines (IL-6, TNF- α)	Downregulates NF- κ B and MAPK pathways	Short plasma half-life, limited tissue accumulation [25]

Theaflavin-3,3'-Digallate: Bioactivities and Challenges

Biological activities

TFDG is a prominent polyphenolic compound derived from black tea, recognized for its potent antioxidant and anticancer properties. Structurally enriched with multiple hydroxyls and gallate groups, TFDG exhibits strong free radical scavenging activity, enabling effective neutralization of reactive oxygen species (ROS) and maintenance of cellular redox homeostasis [18]. This antioxidant capacity is particularly relevant in cancer and chemotherapy associated oxidative stress, where excessive ROS generation contributes to both tumor progression and damage to normal tissues [19].

In vitro studies have demonstrated that TFDG exerts significant antiproliferative and pro-apoptotic effects in a variety of cancer cell lines. Notably, TFDG has been reported to enhance the sensitivity of ovarian cancer cells to chemotherapeutic agents such as cisplatin by modulating intracellular glutathione levels and regulating copper transporter proteins involved in drug uptake and efflux. Through these mechanisms, TFDG may help overcome chemoresistance while allowing lower therapeutic doses of cytotoxic drugs.

Beyond its anticancer activity, TFDG exhibits marked cytoprotective effects in non-malignant cells. Emerging evidence indicates that TFDG can inhibit ferroptosis, a form of iron dependent lipid peroxidation driven cell death, by activating the Nrf2/ GPX4 signalling pathway. This mechanism contributes to the attenuation of oxidative stress, inflammation, and tissue injury, highlighting TFDG's dual role as both a chemosensitizer and a protective antioxidant in cancer therapy contexts [20].

Limitations of free TFDG

Despite its well documented antioxidant and anticancer activities, the therapeutic application of free TFDG is significantly limited by unfavorable pharmacokinetic properties. One of the primary challenges associated with TFDG is its poor aqueous solubility and low intestinal permeability, which restrict efficient absorption following oral administration (table 1). As a polyphenolic compound with high molecular complexity, TFDG undergoes extensive degradation in the gastrointestinal tract and is subject to rapid phase II metabolism, including glucuronidation and sulfation, resulting in low systemic bioavailability [20].

Furthermore, the instability of free TFDG under physiological conditions leads to rapid clearance from circulation, preventing sustained therapeutic concentrations in target tissues. These limitations severely compromise its efficacy as a chemoprotective agent *in vivo*, despite strong

in vitro activity (figure 1). Consequently, the disparity between experimental and clinical outcomes underscores the need for advanced delivery systems capable of protecting TFDG from premature degradation, enhancing absorption, and prolonging systemic retention to enable its successful translation into chemotherapy supportive interventions [21].

β -Chitosan Nanoparticles as Delivery Platforms

β -Chitosan nanoparticles have emerged as highly effective delivery systems in biomedical and pharmaceutical applications due to their favorable physiochemical and biological properties. β -Chitosan is a naturally derived, biodegradable, and biocompatible polysaccharide obtained from the deacetylation of chitin, and it can be readily formulated into nanoparticles using mild techniques such as ionic gelation. This method avoids harsh solvents and high temperatures, thereby preserving the structural integrity and biological activity of encapsulated bioactive compounds. A distinctive feature of β -chitosan is its cationic nature, which enables strong electrostatic interactions with negatively charged biological membranes and mucosal surfaces. This positive surface charge confers mucoadhesive properties that enhance retention time at epithelial barriers and promote paracellular transport, ultimately improving absorption and bioavailability of loaded therapeutics [26].

In the context of drug delivery, β -chitosan nanoparticles offer multiple advantages, particularly for labile phytochemicals such as tea polyphenols. Encapsulation with β -chitosan matrices protects sensitive compounds from chemical and enzymatic degradation under physiological conditions, thereby extending their stability and circulation time. Moreover, β -chitosan nanoparticles facilitate improved cellular uptake through endocytosis and allow for controlled and sustained release of encapsulated agents, maintaining therapeutic concentrations over prolonged periods. Importantly, β -chitosan-based systems can be engineered to respond to specific microenvironmental cues such as acidic pH, elevated oxidative stress, or inflammatory signals, enabling preferential drug release at sites of chemotherapy induced tissue damage. Accumulating evidence indicates that nanoencapsulation significantly enhances the bioavailability and functional efficacy of tea polyphenols, including TFDG analogues, supporting the potential of β -chitosan nanoparticles as versatile carriers for chemoprotective interventions [27].

Encapsulation of Theaflavin-3'-3'- Digallate in β -Chitosan Nanoparticles

The encapsulation of TFDG within β -chitosan-based nanoparticle systems represents a promising strategy to overcome the intrinsic pharmacokinetic limitations of free TFDG and to enhance its therapeutic potential as a chemoprotective agent (figure 2). By integrating the bioactive properties of TFDG with the favorable delivery characteristics of β -chitosan nanoparticles, this approach aims to improve intestinal absorption, stability, and targeted bioavailability while preserving the compound's biological activity [28].

Enhancement of Bioavailability

One of the major challenges associated with oral administration of TFDG is its poor intestinal permeability, which significantly limits systemic exposure. Encapsulation of TFDG in β -chitosan polypeptide nanocomplexes has been shown to markedly enhance intestinal transport through multiple complementary mechanisms. The positive surface charge of β -chitosan facilitates electrostatic interactions with negatively charged epithelial membranes and mucosal surfaces, promoting prolonged residence time in the gastrointestinal tract. Additionally, β -chitosan nanoparticles can transiently open tight junctions between epithelial cells, thereby enhancing paracellular transport of encapsulated polyphenols [29].

Nanoparticle mediated delivery also improves the solubility and dispersibility of TFDG in the gastrointestinal milieu, reducing aggregation and precipitation that typically hinder absorption of free polyphenols. Experimental studies using *in vitro* intestinal models and *ex vivo* permeability assays have demonstrated significantly increased

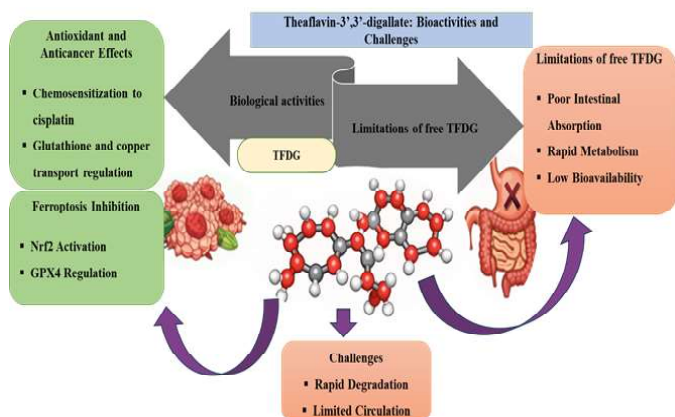


Figure 1: Bioactives and limitations of TFDG, highlighting its antioxidant and anticancer effects alongside challenges of poor bioavailability and rapid metabolism

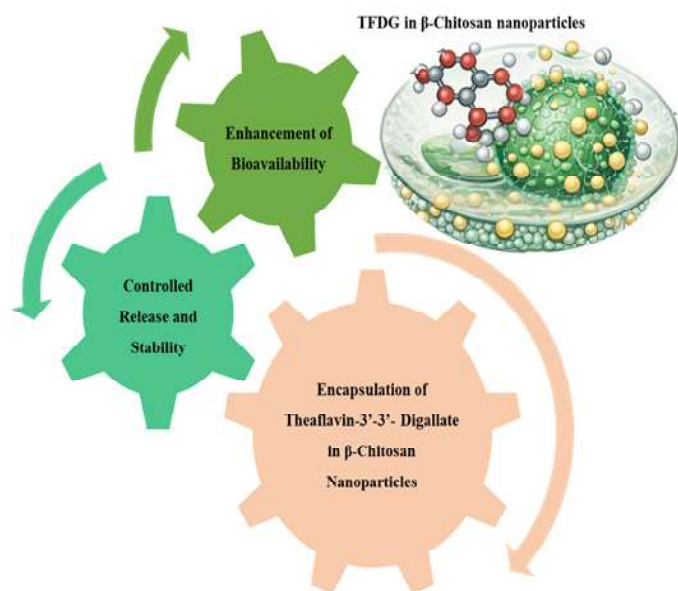


Figure 2: β -Chitosan nanoparticles as delivery platforms for TFDG. The schematic illustrates the role of β -chitosan nanoparticles in enhancing TFDG stability, intestinal absorption, controlled release and targeted delivery, thereby improving bioavailability and chemoprotective efficacy

transepithelial transport of β -chitosan encapsulated tea polyphenols compared to their free counterparts. Enhanced bioavailability not only increases systemic exposure but also enables TFDG to exert its antioxidant and cytoprotective effects at therapeutically relevant concentrations. These improvements are particularly important for applications aimed at mitigating chemotherapy induced toxicity, where sustained systemic availability is essential for effective protection of normal tissues [30].

Controlled Release and Stability

In addition to improving absorption, encapsulation within β -chitosan nanoparticles provides substantial protection against the premature deg-

radation of TFDG in harsh gastrointestinal environments (table 2). Free TFDG is highly susceptible to acidic pH, enzymatic hydrolysis, and oxidative degradation, leading to rapid loss of biological activity before reaching systemic circulation. β -chitosan nanoparticles act as a physical barrier, shielding TFDG from gastric acidity and digestive enzymes, thereby preserving its structural integrity during transit through the gastrointestinal tract.

Controlled and sustained release is another critical advantage of β -chitosan based encapsulation systems. The polymeric matrix allows gradual diffusion of TFDG over time, preventing rapid clearance and maintaining stable plasma concentrations. Furthermore, β -chitosan nanoparticles can be engineered to respond to environmental triggers such as pH changes, oxidative stress, or inflammatory conditions, enabling preferential release of TFDG at sites of tissue injury commonly associated with chemotherapy. This targeted release minimizes off target effects and maximizes therapeutic efficiency. Collectively, enhanced stability and controlled release contribute to higher effective concentrations of bioactive TFDG in systemic circulation and target tissues. Such shielded delivery systems hold significant promise for improving the clinical applicability of TFDG, positioning β -chitosan nanoparticles as a powerful platform for polyphenol based chemoprotective therapies [31].

The therapeutic performance of TFDG- β -CS-NPs is strongly influenced by their physicochemical characteristics, which determine stability, bioavailability, cellular uptake, and release behavior. An optimized formulation is generally expected to exhibit a particle size ranging between 100 and 250 nm, facilitating efficient intestinal absorption, prolonged circulation, and enhanced cellular internalization [32]. A polydispersity index (PDI) below 0.30 is desirable to ensure formulation homogeneity and reproducibility, while a positive zeta potential in the range of +20 to +40 mV contributes to colloidal stability through electrostatic repulsion and enhances interaction with negatively charged biological membranes [33]. Encapsulation efficiency values exceeding 70% and loading capacities between 5–20% are considered favorable for achieving sustained therapeutic concentrations of TFDG [34]. Furthermore, β -CS-NPs are expected to exhibit pH-responsive release behavior, characterized by minimal drug leakage under physiological pH conditions (pH 7.4) and accelerated release under mildly acidic environments (pH 5.5–6.5), such as inflamed tissues or sites of chemotherapy-induced injury [35]. Such controlled release profiles may enable prolonged antioxidant protection while minimizing premature drug loss. Collectively, these physicochemical attributes represent important quality parameters that should be considered during formulation development and optimization of TFDG- β -CS-NP systems intended for

Table 2: Advances of β -Chitosan Nanoparticles for TFDG Delivery

Feature	Description	Impact on TFDG Delivery / Chemotherapy Protection
Biocompatibility & Biodegradability	Derived from chitin; safe for biomedical use	Reduces systemic toxicity; suitable for repeated dosing [36].
Mucoadhesiveness & Positive Charge	Interacts with epithelial surfaces	Enhances intestinal absorption and paracellular transport [37].
Protection from Degradation	Encapsulation shields TFDG from gastric acid, enzymes	Improves stability and systemic bioavailability [38].
Controlled / Sustained Release	Polymer matrix allows gradual diffusion	Maintains therapeutic plasma concentrations over time [39].
Targeted Delivery	Can be engineered to release in acidic, oxidative, or inflamed tissues	Minimizes off-target effects; protects organs during chemotherapy [40].
Enhanced Cellular Uptake	Nanoparticles facilitate endocytosis	Ensures efficient delivery of TFDG to target tissues [41].
Potential Synergy with Chemotherapy	Preserves anticancer activity while protecting healthy tissue	Reduces chemotherapy-induced toxicity in liver, intestine, and kidney [42].

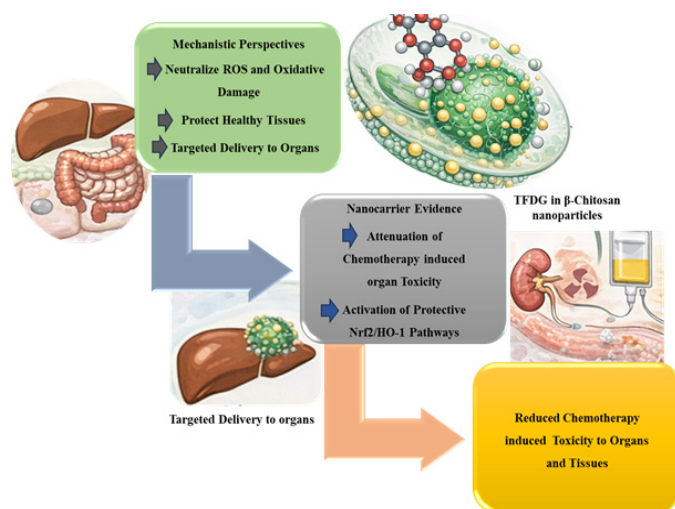


Figure 3: Mechanisms by which TFDG-loaded β -CS-NPs mitigate chemotherapy induced toxicity by reducing oxidative stress and protecting normal tissues

chemoprotective applications.

In addition to particle size, zeta potential, encapsulation efficiency, and release kinetics, comprehensive solid-state characterization is essential for confirming the structural integrity of TFDG- β -CS-NPs. X-ray diffraction (XRD) provides valuable information regarding the physical state of encapsulated TFDG by determining whether the crystalline drug has been converted into a molecularly dispersed amorphous form within the β -chitosan polymeric matrix. Such amorphization is generally associated with improved solubility, dissolution behavior, and formulation stability. Complementary Fourier-transform infrared spectroscopy (FTIR) analysis is equally important for verifying the chemical compatibility between TFDG and β -chitosan. Characteristic shifts in hydroxyl, amino, and carbonyl absorption bands can confirm successful intermolecular hydrogen bonding and electrostatic interactions while simultaneously excluding undesirable chemical modifications of the β -chitosan backbone during nanoparticle fabrication. Together, XRD and FTIR serve as essential analytical tools for validating formulation integrity, ensuring batch-to-batch reproducibility, and supporting future pharmaceutical quality-control requirements.

Potential Roles in Mitigating Chemotherapy Toxicity

Encapsulation of TFDG within β -chitosan nanoparticles offers a mechanistically attractive strategy for reducing chemotherapy associated toxicity while preserving anticancer efficacy. Chemotherapeutic agents commonly induce excessive production of ROS, triggering oxidative stress, inflammation, mitochondrial dysfunction, and subsequent damage to normal tissues such as the liver, intestine, kidney, and bone marrow [29, 42]. TFDG, owing to its strong antioxidant and metal chelating properties, has the potential to neutralize chemotherapy induced free radicals, restore redox homeostasis, and suppress downstream inflammatory signaling in non-malignant cells. When delivered via β -chitosan nanoparticles, these protective effects may be further amplified through enhanced tissue targeting, prolonged systemic circulation, and preferential accumulation in organs highly exposed to chemotherapeutic stress, particularly the gastrointestinal tract and liver (figure 3).

An important consideration in the application of TFDG- β -CS-NPs as chemoprotective agents is their potential interaction with the cytotoxic mechanisms of conventional chemotherapeutic drugs. Several anticancer agents, particularly anthracyclines such as doxorubicin and daunorubicin, partially exert their antitumor activity through the generation of reactive oxygen species (ROS), raising concerns that potent

antioxidants may inadvertently attenuate therapeutic efficacy [43]. In contrast, other classes of chemotherapeutics, including antimetabolites such as 5-fluorouracil and methotrexate, primarily target DNA synthesis and cell proliferation pathways, making them less dependent on oxidative mechanisms for tumor cell killing [44]. Platinum-based agents such as cisplatin possess a dual mechanism involving both DNA crosslinking and oxidative stress induction, while taxanes predominantly interfere with microtubule dynamics and mitotic progression [45]. Consequently, the influence of TFDG may vary according to the chemotherapeutic class employed. Emerging evidence suggests that TFDG exerts biological effects beyond ROS scavenging, including modulation of inflammatory signaling, ferroptosis regulation, mitochondrial function, and cellular stress responses. Furthermore, nanoparticle-mediated delivery may promote controlled and site-specific release, potentially allowing preferential protection of healthy tissues exposed to chemotherapy-induced oxidative injury while minimizing interference with tumor-directed cytotoxicity. Nevertheless, the possibility of tumor protection cannot be completely excluded, and future studies should systematically evaluate the pharmacodynamic interactions between TFDG- β -CS-NPs and different classes of chemotherapeutic agents to establish optimal therapeutic combinations that maximize normal tissue protection without compromising anticancer efficacy.

Although direct *in vivo* evidence for TFDG- β -CS-NPs in chemotherapy toxicity models remains limited, substantial support is provided by related nanocarrier systems. β -chitosan based nanoparticles encapsulating antioxidants or polyphenols have been shown to attenuate drug induced organ toxicity by activating endogenous cytoprotective pathways such as Nrf2/HO-1, reducing lipid peroxidation, and preserving cellular antioxidant defenses. Similarly, nanoparticle formulations of polyphenols such as quercetin and catechins consistently demonstrate superior protection against oxidative and developmental toxicity compared with free compounds. Collectively, these findings support the concept of TFDG- β -CS-NPs as a promising bioshield to mitigate chemotherapy-induced damage [46].

Safety and Toxicity Considerations

Although β -chitosan nanoparticles are widely regarded as safe, biodegradable, and biocompatible, a thorough evaluation of their safety and toxicity profiles is essential before their translation into clinical applications, particularly in the context of chemotherapy adjuvant therapy. Numerous *in vitro* and *in vivo* studies indicate the β -chitosan based nanocarriers exhibit minimal cytotoxicity and toward normal cells while demonstrating selective cytotoxic or sensitizing effects in tumor tissues. This selective behavior is often attributed to the enhanced permeability and retention effect in tumors, differences in cellular uptake mechanisms, and the acidic tumor microenvironment, which can promote preferential nanoparticle accumulation and drug release. However, the toxicological outcomes of β -chitosan nanoparticles are not uniform and depend on multiple physicochemical parameters, including particle size, surface charge, molecular weight, degree of deacetylation, and administered dose.

Smaller nanoparticles with high positive surface charge may exhibit increased cellular uptake but can also induce membrane disruption, mitochondrial stress, or inflammatory responses at higher concentrations. Similarly, repeated or high dose exposure to β -chitosan nanoparticles may lead to accumulation in organs such as the liver, spleen, or kidneys, potentially triggering immune activation or mild organ stress, particularly in compromised physiological conditions. The route of administration further influences safety outcomes, with oral delivery generally showing better tolerability compared to intravenous routes, which demand stricter control over sterility, aggregation, and immunogenicity [47].

When loaded with bioactive compounds such as TFDG, the toxicity profile may be further modulated by altered biodistribution and prolonged systemic retention. While encapsulation often reduces off-target toxicity by enabling controlled release and tissue specific delivery, it also necessitates careful evaluation of long-term exposure effects. Comprehensive toxicological assessments across multiple biological models- in-

cluding acute, sub-chronic, and chronic studies are therefore crucial. Such evaluations should integrate histopathological, biochemical, immunological, and genotoxic endpoints to ensure safety. Overall, while existing evidence supports the favorable safety profile of β -chitosan nanoparticles, systemic and standardized toxicity studies remain indispensable for their responsible clinical translation as chemoprotective nanoplatforms [48].

Future Directions

The encapsulation of TFDG within β -CS-NPs presents a promising strategy to mitigate chemotherapy induced toxicity, yet significant gaps remain that must be addressed to advance this field toward clinical translation. While preliminary *in vitro* studies have demonstrated the potential of TFDG- β -CS-NPs to enhance bioavailability, stability, and antioxidant efficacy, comprehensive *in vivo* validation is essential. Future research should prioritize rigorous *in vivo* studies using relevant animal models of chemotherapy-induced organ toxicity, including hepatic, renal, gastrointestinal, and hematopoietic systems. Such studies would provide critical insights into the biodistribution, pharmacokinetics, and therapeutic efficacy of TFDG- β -CS-NPs under physiological conditions, allowing assessment of their capacity to attenuate systemic oxidative stress and tissue damage during chemotherapeutic regimens [49].

Comparative studies contrasting free TFDG with its nanoparticle encapsulated counterpart are also imperative. Quantitative evaluation of bioavailability, plasma half-life, tissue penetration, and accumulation in target versus non-target organs will clarify the functional advantages of nanoparticle mediated delivery. These analyses can further elucidate whether TFDG- β -CS-NPs achieve therapeutically relevant concentrations at critical sites of chemotherapy induced injury, thereby establishing a foundation for dose optimization and translational relevance. Additionally, mechanistic investigations should focus on the molecular pathways modulated by TFDG- β -CS-NPs in normal and cancerous tissues. Particular attention should be directed toward oxidative stress responsive pathways such as Nrf2/HO-1 and GPX4, as well as inflammatory signaling cascades, to determine how these nanocarriers mediate cellular protection while preserving or enhancing anticancer activity [50].

Safety and toxicity profiling remains a cornerstone for future research. Although chitosan is generally regarded as biocompatible and non-toxic, factors such as nanoparticle size, surface charge, dose, and route of administration can influence both acute and long-term safety outcomes. Systemic evaluation across multiple organ systems, coupled with detailed histopathological, biochemical, immunological, and genotoxic assessments, is required to ensure translational feasibility. Biodistribution studies using advanced imaging and labelling techniques will further provide critical data on nanoparticle accumulation, clearance, and potential off target effects [51].

Despite the encouraging preclinical evidence, several manufacturing and translational challenges remain before TFDG- β -CS-NPs can progress toward clinical application. Although ionic gelation is highly suitable for laboratory-scale preparation because of its simplicity and mild processing conditions, reproducible large-scale manufacturing under Good Manufacturing Practice (GMP) conditions remains technically demanding. Industrial production requires precise control over particle size distribution, encapsulation efficiency, polymer molecular weight, mixing dynamics, and batch-to-batch reproducibility. Sterilization also represents a significant challenge because conventional techniques such as steam autoclaving may induce depolymerization of β -chitosan, while gamma irradiation can alter polymer molecular weight and compromise nanoparticle stability. Consequently, aseptic manufacturing or carefully optimized low-temperature sterilization approaches may be more appropriate for preserving nanoparticle integrity. Furthermore, long-term storage stability requires optimization of freeze-drying (lyophilization) protocols to prevent nanoparticle aggregation and preserve drug loading. Appropriate cryoprotectants such as trehalose, sucrose, or mannitol should be incorporated to maintain particle size, surface charge, and encapsulation efficiency following reconstitution. Addressing these pharmaceutical manufacturing considerations will be essential for successful regulatory approval and clinical translation of TFDG- β -CS-NPs.

Finally, integration of TFDG- β -CS-NPs with complementary che-

motherapeutic regimens offers a fertile area of investigation. Studies should explore how these nanoparticles interact with conventional anticancer drugs, examining potential synergistic or antagonistic effects, as well as strategies to minimize interference with chemotherapeutic efficacy. Optimization of formulation parameters, including particle size, surface modification, and controlled release kinetics, may enhance targeting to vulnerable tissues and further reduce systemic toxicity. Overall, a multidisciplinary approach combining nanotechnology, pharmacology, oncology, and toxicology is essential to translate TFDG- β -CS NP systems from bench to bedside, ultimately providing a safe, effective, and clinically viable strategy to shield healthy tissues from the deleterious effects of chemotherapy.

Conclusion

TFDG- β -CS-NPs represents a promising strategy to enhance the bioavailability, stability, and targeted delivery of this potent polyphenol. By combining TFDG's antioxidant and cytoprotective effects with the favorable properties of β -chitosan nanoparticles such as controlled release, mucoadhesion, and biocompatibility - this approach holds significant potential to mitigate chemotherapy induced toxicity while preserving anticancer efficacy. Although further *in vivo* validation, mechanistic studies, and comprehensive safety evaluations are required, TFDG- β -CS-NPs systems offer a novel, biologically inspired platform for improving chemotherapy tolerance and advancing polyphenol based supportive therapies in oncology.

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