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Review

Tocotrienol as a Potential Treatment for Drug-Induced Liver Injury

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Abstract

Oxidative stress and inflammation are widely recognized as key mechanisms in the pathogenesis of Drug-Induced Liver Injury (DILI). Meanwhile, preclinical studies have demonstrated that tocotrienols (T3s), members of the vitamin E family, possess significant antioxidant and anti-inflammatory properties, suggesting a potential hepatoprotective role in various liver disorders. Clinical trials investigating Non-Alcoholic Fatty Liver Disease (NAFLD), a condition that shares important pathophysiological features with DILI, have also reported favorable outcomes associated with T3 supplementation. Notably, the overlap between the established mechanisms of T3s and the underlying pathophysiology of DILI provides a strong rationale for exploring the therapeutic potential of T3s in this context. Emerging evidence from studies on NAFLD further supports this approach, considering the common mechanistic pathways involved. Accordingly, this review aims to comprehensively evaluate current preclinical and clinical evidence on T3s in relation to DILI, elucidate the proposed mechanisms of action of this class of vitamin E analog, and identify key gaps in the literature that warrant further investigation.

Keywords: antioxidants; chemical and drug induced liver injury; hepatoprotection; inflammation; oxidative stress; tocotrienols; vitamin E

1. Introduction to Drug-Induced Liver Injury (DILI)

The liver is the largest organ and plays a crucial role in the metabolism and elimination of drugs from the body. Correspondingly, the detoxification of drugs and xenobiotics by metabolizing enzymes in the liver is a crucial process for maintaining homeostasis [1]. As such, Drug-Induced Liver Injury (DILI) is defined as liver dysfunction caused by exposure to certain medications or dietary supplements that can damage hepatocytes and other liver cells [2]. Following this, DILI remains a significant clinical concern and is a major contributor to liver-related morbidity and mortality worldwide [3]. It can also imitate all forms of acute or chronic liver disease [4]. Furthermore, the incidence of DILI is estimated at approximately 19 cases per 100,000 individuals per year, accounting for a substantial proportion of hospitalizations related to jaundice and hepatitis [5]. However, the rate and severity of DILI vary between drugs, suggesting that drug-specific properties influence the risk of developing DILI [6]. A person's susceptibility to a possible hepatotoxic drug is influenced by various factors. For instance, elderly individuals are at a higher risk of developing DILI due to prolonged medication use and heavy alcohol consumption. Additionally, women are more prone to developing autoimmune hepatitis, which can result in liver damage [2,7]. Common culprits implicated in DILI include widely used drugs such as acetaminophen (APAP) and HMG-CoA reductase inhibitors (statins) [5]. Anticancer therapies are also recognized to elevate the risk of DILI [8]. The frequent occurrence and potential severity of DILI underscore the urgent need for effective strategies for its prevention and management.

There are two types of DILI: idiosyncratic (unpredictable) and intrinsic (direct) hepatotoxicity. Intrinsic hepatotoxicity occurs as a result of exposure to foreign agents that are directly toxic to the liver. This type of injury is typically dose-dependent and develops within a short timeframe, usually one to five days after drug exposure. However, such cases are relatively rare, occurring in approximately 1 out of every 2000 patient exposures [9]. An example of intrinsic DILI is acetaminophen (APAP) hepatotoxicity, which causes almost 50% of acute liver failure [7,10]. Direct hepatotoxicity occurs when individuals consume slightly higher-than-recommended doses of APAP, resulting in elevated alanine aminotransferase (ALT) levels [11]. Both APAP and hydrogen peroxide (H_2O_2) have been demonstrated to increase intracellular reactive oxygen species (ROS) and lipid peroxidation (LPO), which are key markers of oxidative stress and liver injury [12].

Idiosyncratic hepatotoxicity is an unpredictable and uncommon form of liver injury that is not dose-dependent and is unrelated to the pharmacological action of the drug. This type of DILI is typically influenced by factors such as age, sex, obesity, alcohol consumption, diabetes mel-

litus, and underlying chronic liver disease. Notably, the use of multiple medications (e.g., isoniazid, flucloxacillin, halothane, and amoxicillin), known as polypharmacy, increases the risk of DILI, particularly in older individuals [2,13]. However, its overall incidence remains relatively low among individuals exposed to it. This limitation poses a significant challenge for further research, as the small number of affected patients reduces the feasibility of conducting large-scale clinical studies.

2. Pathophysiology of Drug-Induced Liver Injury

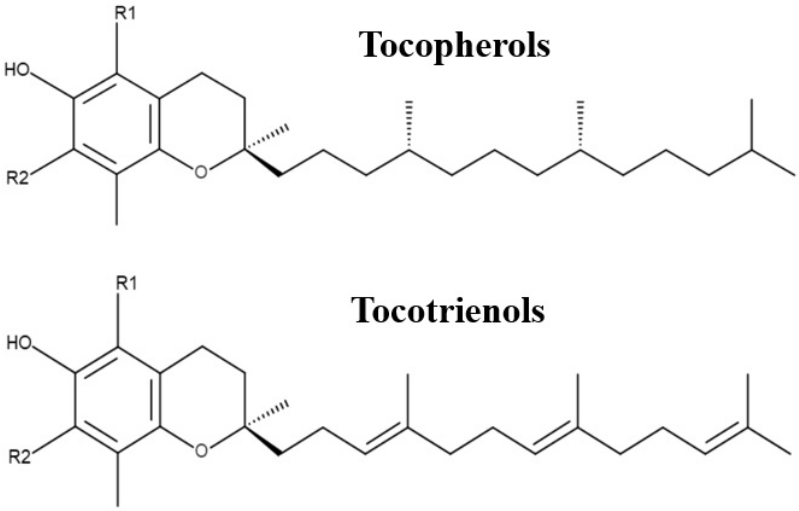
DILI is widely recognized as a significant cause of liver impairment and acute liver failure, making it essential to understand the mechanisms underlying this condition. The liver plays a central role in drug metabolism, and the accumulation of reactive metabolites makes it particularly susceptible to toxicity. The development of DILI primarily results from liver cell death through necrosis, apoptosis, and necroptosis, as well as from oxidative stress and mitochondrial dysfunction [14].

Necrosis may result from oncosis, a condition characterized by the disruption of ion and water homeostasis within the cell. This disruption leads to cellular swelling and, ultimately, the rupture of the plasma membrane, resulting in the release of intracellular contents, including cytotoxic and pro-inflammatory factors, into the extracellular space. Depletion of adenosine triphosphate (ATP), caused by impaired mitochondrial oxidative phosphorylation, further promotes cell death. Furthermore, the release of intracellular components, such as damage-associated molecular patterns (DAMPs), into the surrounding environment can trigger inflammatory responses. This process distinguishes necrosis from apoptosis, which is typically non-inflammatory [15]. Accordingly, diclofenac is metabolized in the liver, where phase I enzymes convert it into metabolites such as 5-hydroxydiclofenac. This metabolite undergoes further oxidation to form electrophilic quinone imine intermediates. These reactive species covalently bind to mitochondrial proteins, resulting in structural and functional impairment of the organelles. The depletion of mitochondrial glutathione (mtGSH) further enhances oxidative stress within hepatocytes, disrupting calcium homeostasis and compromising ATP synthesis. Diclofenac causes dose-dependent mitochondrial collapse, ATP depletion, and necrotic cell death, especially when glutathione (GSH) levels are depleted, playing a critical role in the development of diclofenac-induced hepatotoxicity [16].

Apoptosis is a form of programmed cell death that can occur through either the extrinsic or intrinsic pathway. The extrinsic apoptosis pathway is triggered by external signals that activate death receptors on the surface of cells, such as First Apoptosis Signal receptor (FAS) Ligand (FASL), TNF-Related Apoptosis-Inducing Ligand Receptor 1 and 2 (TRAIL-R1/R2), and Tumor Necrosis Factor Receptor 1 (TNFR1). These receptors bind to their respective ligands, TRAIL and TNF, which leads to the formation of a signaling complex that activates caspase-8. In the liver, hepatocytes naturally express high levels of these death receptors. During an immune response, CD8+ cytotoxic T cells become activated and induce apoptosis in hepatocytes by expressing FASL, which binds to FAS. This interaction activates caspase-8 and initiates the apoptotic process [17]. Flucloxacillin is metabolized in the body and can covalently bind to host proteins, forming drug-protein adducts. These adducts are subsequently broken down into peptides, some of which contain fragments of the flucloxacillin molecule. As such, the immune system may mistakenly recognize these drug-derived peptides as harmful, triggering cytotoxic CD8+ T cells to attack hepatocytes. This immune response can lead to liver injury and inflammation, potentially through the activation of the extrinsic apoptotic pathway, in which death receptor signaling induces programmed cell death in hepatocytes [18].

The intrinsic apoptotic pathway is activated in a dose-dependent manner following exposure to the drug. Most drugs are metabolized in the liver by the cytochrome P450 enzyme system, which produces reactive metabolites. These metabolites can covalently bind to mitochondrial proteins, leading to the accumulation of ROS within the mitochondria. In particular, mitochondrial dysfunction is a critical component of DILI pathophysiology. Increased ROS production contributes to mitochondrial damage and initiates a cellular stress response aimed at mitigating the injury. This response can trigger mitochondrial outer membrane permeabilization (MOMP), resulting in the release of cytochrome c and the activation of caspase-9, ultimately leading to apoptosis [19]. Drug metabolites and the associated oxidative stress impair normal mitochondrial function, leading to cellular energy depletion and, eventually, cell death [20].

The onset of DILI often begins with hepatic drug metabolism, which can produce reactive metabolites that directly damage liver cells. This damage may occur when these metabolites form covalent bonds with essential cellular macromolecules, disrupting normal cellular functions. A well-known example is the hepatic metabolism of APAP, which generates the toxic intermediate N-acetyl-p-benzoquinone imine (NAPQI) [5]. It is well known that an overdose of APAP can cause liver injury. At therapeutic doses, APAP is primarily metabolized in the liver through glucuronidation and sulfation pathways. A smaller portion is metabolized by cytochrome P450 enzymes to produce NAPQI. Under normal conditions, NAPQI is detoxified by conjugation with GSH. However, in overdose situations, GSH stores become depleted, allowing NAPQI to accumulate. This accumulation leads to oxidative stress due to the excessive production of ROS, which contributes to liver failure [21]. The elevation of ROS can trigger LPO, compromising the structural integrity of cellular membranes



Tocochromanol type	R1	R2
α-Tocopherol	CH_3	CH_3
α-Tocotrienol	CH_3	CH_3
β-Tocopherol	CH_3	H
β-Tocotrienol	CH_3	H
γ-Tocopherol	H	CH_3
γ-Tocotrienol	H	CH_3
δ-Tocopherol	H	H
δ-Tocotrienol	H	H

Fig. 1. The chemical structures of tocopherols and tocotrienols.

[20]. Consistent with this, animal studies on APAP-induced liver injury have presented elevated LPO levels [22]. Mitochondrial dysfunction has also been directly implicated in the pathogenesis of APAP-induced liver injury [23].

Necroptosis is a regulated form of necrotic cell death that occurs when apoptosis is blocked, often triggered by TNF-α binding to TNFR1. Normally, this activates caspase-8 and triggers apoptosis, but without caspase-8, the pathway shifts. Receptor-Interacting Serine/Threonine-Protein Kinase 1 (RIPK1) and Receptor-Interacting Serine/Threonine-Protein Kinase 3 (RIPK3) form the necrosome, activating Mixed Lineage Kinase Domain-Like Protein (MLKL), causing disruption of plasma membranes through pore formation. This causes ion leakage, swelling, rupture, and the release of intracellular contents, such as DAMPs, which activate immune responses and promote inflammation, making necroptosis both a cell death and an immune-activating process [24]. In certain cases, DILI may involve an immune-mediated component, where the immune system recognizes drug metabolites or altered liver proteins as foreign. This recognition can trigger the infiltration of immune cells into liver tissue, exacerbating hepatic injury.

3. Overview of Tocotrienol

Tocopherols and Tocotrienols (T3s) are two groups of vitamin E compounds that occur naturally in various plant sources (Fig. 1). T3s are reported in a range of cereals and vegetables, including palm oil, rice bran oil, coconut oil, barley germ, wheat germ, and annatto. Among these, palm oil and rice bran oil are considered among the richest natural sources of vitamin E, particularly T3s. In addition to these, T3s are also present in other plant-based sources such as rapeseed oil, oats, hazelnuts, maize, olive oil, buckthorn berries, rye, flaxseed oil, poppy seed oil, and sunflower oil [25].

Both T3s and tocopherols are fat-soluble and share a similar structural framework, consisting of a chromanol ring and a side chain attached at the C-2 position (Fig. 1). The primary difference in their side chains is that tocopherols have saturated phytyl tails, whereas T3s possess unsaturated isoprenoid side chains with three double bonds. This structural variation enables T3s to penetrate tissues with saturated fatty layers more effectively, such as the brain and liver. Moreover, T3s exhibit greater dispersion within lipid membranes, contributing to their superior biological activities [26,27].

T3s are the primary phytonutrients present in palm oil and can be observed in a specific fraction of palm oil known as the T3-Rich Fraction (TRF). TRF comprises mostly T3s (80%), which are alpha (α), beta (β), gamma (γ), and delta (δ) T3s, as well as 20% tocopherols [28]. T3s have been reported to exhibit a range of beneficial biological activities, including anti-thrombotic, antioxidant, neuroprotective, cardioprotective, and immunomodulatory effects. It also exhibits anticancer effects by apoptosis, anti-angiogenesis, anti-proliferative, and immunoregulation. Experimental studies comparing the antioxidant and free radical scavenging actions of vitamin E isomers have demonstrated that T3s display enhanced antioxidant, neuroprotective, and anticancer properties [29]. Among the T3 isoforms, δ-T3 is regarded as the most biologically active form of vitamin E [3]. In addition, the unique structural features of T3s may facilitate more efficient targeting of liver tissue, potentially enhancing their therapeutic efficacy compared to other forms of vitamin E [3]. Since oxidative stress plays a key role in the onset and progression of DILI, the enhanced antioxidant potential of T3s makes them promising candidates for mitigating this component of liver damage [30].

4. Hepatoprotective Mechanisms of Tocotrienols Relevant to Intrinsic DILI

T3s are known for their strong antioxidant activity, and studies indicate that they may be more effective than tocopherols in certain biological systems [31]. These compounds can inhibit the production of free radicals and the process of LPO, which are critical events in the progression of liver injury [32]. α-T3 has been proven to exhibit greater antioxidant activity than α-tocopherol in rat liver microsome studies [33]. The strong antioxidant capacity of T3s provides a direct means of reducing oxidative damage associated with DILI. Through the efficient removal of ROS, T3s help protect critical cellular structures from injury caused by toxic drug metabolites.

T3s exhibit antioxidant activity through both direct scavenging of ROS and indirectly enhancing the expression of antioxidant enzymes such as Superoxide Dismutase (SOD) and NAD(P)H quinone oxidoreductase 1 (NQO1). These enzymes help neutralize ROS and prevent oxidative damage. Their expression is regulated by the nuclear factor erythroid 2-related factor 2 (Nrf2), which plays a central role in the cellular antioxidant response. Under oxidative stress, Nrf2 is activated and translocates to the nucleus, where it binds to Antioxidant Response Elements (AREs) in the DNA to promote the transcription of genes encoding antioxidant and detoxifying enzymes, including SOD, NQO1, glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1). Therefore, Nrf2 activates the expression of antioxidant enzymes, which then conduct the antioxidant defense mechanisms. This coordinated response helps the cell restore redox balance and protect against oxidative stress [34–37]. Collectively, these findings suggest that T3s may enhance the liver's natural ability to defend itself against the harmful effects of drugs.

In addition to their antioxidant effects, T3s also have strong anti-inflammatory properties [38]. They have been proven to reduce the expression of major inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) [39]. By lowering the levels of pro-inflammatory cytokines and interfering with related signaling pathways, T3s may help reduce the inflammatory response that exacerbates DILI. Hence, managing inflammation is crucial for slowing the progression and reducing the severity of liver damage. At the same time, the anti-inflammatory effects of T3s may play a significant role in this process.

Endoplasmic reticulum (ER) stress occurs when the protein-folding capacity of the ER becomes saturated, leading to the accumulation of misfolded proteins and the activation of the unfolded protein response (UPR). Prolonged ER stress triggers hepatocyte apoptosis via pro-apoptotic mediators such as CCAAT/Enhancer-Binding Protein (C/EBP) homologous protein (CHOP). T3s exert protective effects by downregulating CHOP expression and modulating key Unfolded Protein Response (UPR) pathways, including PKR-like Endoplasmic Reticulum Kinase (PERK), Inositol-Requiring Enzyme 1 alpha (IRE1α), and Activating Transcription Factor 6 (ATF6), reducing ER stress-induced damage. By inhibiting ER stress, T3s also suppress the activation of hepatic stellate cells (HSCs), which are responsible for collagen deposition and the progression of liver fibrosis [40,41], a common manifestation of DILI.

T3s have also been demonstrated to affect lipid metabolism and support the stability of cellular membranes. They can reduce triacylglycerol levels in hepatocytes, contributing to healthier lipid profiles within the liver [42]. Their unsaturated side chain allows them to penetrate and spread efficiently within cell membranes, which helps maintain membrane integrity during cellular stress [43]. Therefore, preserving membrane structure and regulating lipid metabolism are essential for proper hepatocyte function, particularly in the context of DILI.

Recent findings indicate that T3s may influence the expression of microRNAs (miRNAs), which are small non-coding RNA molecules involved in regulating gene expression. In individuals with non-alcoholic fatty liver disease (NAFLD), both δ-T3 and α -tocopherol have been observed to suppress miRNAs associated with hepatic fat accumulation, insulin resistance, oxidative stress, inflammation, and programmed cell death [44]. Notably, δ-T3 appears to exert a stronger regulatory effect on specific miRNAs associated with inflammation and apoptosis [44]. Moreover, T3s' ability to modulate miRNA activity highlights their potential to regulate cellular responses to DILI at the genetic level, which may offer significant therapeutic benefits.

5. Potential Role of Tocotrienols in Mitigating Idiosyncratic DILI (iDILI)

Idiosyncratic Drug-Induced Liver Injury (iDILI) arises from a complex interplay of drug-induced hepatocellular stress, activation of immune pathways, and host susceptibility factors such as human leukocyte antigen (HLA) alleles [45,46]. Many hepatotoxic drugs generate mitochondrial ROS and LPO, processes that amplify damage signals through the release of DAMPs. Accordingly, these signals engage innate immune sensors, promote cytokine release, and facilitate adaptive immune activation, establishing a foundation for immune-mediated liver injury [47].

Redox balance plays a key regulatory role in this process. Meanwhile, Nrf2, a central transcription factor, suppresses LPO and maintains antioxidant defenses [48]. T3s, members of the vitamin E family, act as potent chain-breaking antioxidants and activate Nrf2-associated pathways. In hepatic and metabolic models, they upregulate protective enzymes such as HO-1 and NQO1, limiting oxidative stress and the generation of DAMPs [49].

Once released, DAMPs are recognized by hepatic pattern recognition receptors such as Toll-Like Receptor 4 (TLR4). This engagement activates Nuclear Factor kappa-

light-chain-enhancer of activated B cells (NF-κB) signaling, driving the production of pro-inflammatory cytokines including TNF-α and IL-1β, which prime T-cell responses and contribute to iDILI pathogenesis [47]. T3s have been demonstrated to inhibit TLR4/MyD88–NF-κB signaling and lower cytokine production, reducing the inflammatory environment required for disease initiation [50].

DAMPs can also activate the NLRP3 inflammasome, amplifying IL-1β-driven inflammation and immune injury [48]. δ- and γ-T3s directly attenuate inflammasome activation by reducing caspase-1 cleavage and IL-1β release in preclinical models, providing a plausible brake on this inflammatory amplifier [51]. Beyond these pathways, drug-induced stress in the ER and mitochondria further activates c-Jun N-terminal kinase (JNK), NF-κB, and cell-death cascades [48]. T3s mitigate ER and mitochondrial stress, with δ-T3 regulating CHOP-mediated ER stress signaling *in vitro*, dampening downstream inflammatory responses [49,52]. Although most of these findings are outside the context of iDILI, they provide mechanistic insights that are highly relevant to its pathogenesis.

Adaptive immunity also shapes iDILI outcomes. In many cases, T cells recognize drug-modified peptides in an HLA-restricted manner. However, progression to injury depends on cytokine signals and immune checkpoint tone [53]. Notably, T3s can downregulate Programmed Death-Ligand 1 (PD-L1) and enhance T-cell activity, suggesting possible effects on immune resolution, though the balance between tolerance and injury remains uncertain [54,55].

Some iDILI cases present as cholestatic injury due to bile salt export pump (BSEP) inhibition or immune-mediated disruption [56,57]. While T3s have not been demonstrated to directly upregulate BSEP in humans, their antioxidant and anti-inflammatory effects may indirectly support transporter function [56].

MicroRNAs are essential regulators of DILI biology, modulating Nrf2, NF-κB, apoptosis, and bile acid pathways [48]. δ-T3 has been introduced in NAFLD patients to reduce circulating miR-34a and influence networks involving miR-122, correlating with improved liver biochemistry. Although direct studies in iDILI are lacking, these findings suggest that T3s may modulate stress- and immune-related pathways highly relevant to iDILI pathogenesis [44,58].

6. Emerging Roles of miRNA and Exosome Pathways in Tocotrienol Hepatoprotective Mechanisms

MicroRNAs, particularly miR-122 and miR-34a, are key regulators and biomarkers in human DILI, with elevated circulating and exosome-associated levels linked to hepatocyte injury and inflammation [59–61]. Hepatocyte-derived exosomes deliver miR-122 to macrophages and Kupffer cells, promoting a pro-inflammatory shift through NF-κB and related pathways in models of liver ischemia-reperfusion and alcohol-induced injury. Inhibition of miR-122 reduces this inflammatory response [62,63]. Similarly, foundational studies have indicated that circulating/exosomal miR-122 is a sensitive indicator of hepatocyte injury [64].

The miR-34a-SIRT1 axis represents a central stress-response pathway. Moreover, miR-34a represses SIRT1, whereas higher SIRT1 levels protect against hepatic stress and injury [65]. DILI and iDILI pathogenesis are therefore strongly influenced by miR-122/miR-34a signaling and exosome-mediated communication [59,63,64]. Additionally, δ-T3 has demonstrated the ability to lower circulating miR-34a in humans, consistent with relieving SIRT1 repression, and may also plausibly affect exosome signaling [44,63,66–68]. However, no study has directly assessed whether T3s lower miR-122 in DILI or iDILI [44]. Although the evidence is strongest in NAFLD/metabolic liver disease, considering that miR-122 is also elevated in DILI, it is mechanistically plausible that T3s could also suppress it in that context [44].

T3s have been presented to influence exosome biology in oncology and immune settings. γ-T3 disrupts lipid rafts involved in exosome signaling, while δ-T3 reduces PD-L1 glycosylation and exosomal PD-L1 release, suggesting potential effects on exosomal cargo and communication [66,67,69]. Whether δ-T3 directly remodels hepatocyte exosome output during iDILI has not yet been demonstrated. Although no direct human clinical trials in DILI or iDILI involving T3s have yet been conducted, all the mechanistic examples described above are convincing and support the need for targeted preclinical and early clinical investigation.

7. Preclinical Evidence: Tocotrienol in Animal Models of Drug-Induced Liver Injury

In vitro hepatocyte studies indicate T3s protect against DILI. Using TGF-α transgenic murine hepatocyte (TAMH) cell lines exposed to APAP and H_2O_2, both α-T3 and α-tocopherol demonstrated cytoprotective effects [12]. Two protocols were assessed: concurrent exposure and 24-hour pre-treatment. In particular, α-T3 was more effective than α-tocopherol in reducing intracellular ROS, with γ-T3 also effective at lower concentrations. Meanwhile, pre-treatment highlighted α-T3's potential as a preventative agent. Both compounds inhibited apoptosis and promoted liver regeneration markers. However, α-T3 presented higher uptake. These findings strongly support T3s as direct hepatoprotective agents against oxidative stress–induced hepatocellular injury [12].

In vivo studies using animal models have provided critical evidence for the protective effects of T3s against DILI. A summary of these findings is presented in Table 1 (Ref. [12,70–81]). Early studies in the 1990s first demonstrated T3s' potential in DILI and hepatocarcinogenesis [70,71]. In one study, male rats were exposed to 2-acetylaminofluorene (AAF) for 20 weeks. AAF markedly

Table 1. Summary of preclinical studies on tocotrienols in drug-induced liver injury models.

Study (year)	Animals (cells) used & liver injury model	Tocotrienol form & dose	Main outcomes	Conclusion
Wan Ngah *et al*. (1991) [71]	Male *Rattus norwegicus* rats. Liver injury induction → 2-acetylaminofluorene (AAF), administered to the rats at a concentration of 0.02% in their basal diet.	A γ-tocotrienol-enriched fraction (consisting of 80% γ-tocotrienol and 20% α- and β-tocotrienol) was used. This was administered to the rats as a supplement in their diet at a dose of 0.7 mg per rat per day.	• AAF + Tocotrienols co-administration + → ↓liver cell damage + ↓nuclear irregularity. • AAF + Tocotrienol co-administration → significantly ↓plasma GGT (12 and 20 weeks) & ↓UDP-GT activity (20 weeks). Liver microsomal GGT activity ↓slightly.	Tocotrienols offered some protection against AAF-induced liver changes and reduced the severity and extent of neoplastic transformation in the rat livers.
Rahmat *et al*. (1993) [70]	Male *Rattus norwegicus* rats. Liver injury induction → diethylnitrosamine (DEN) and acetylaminofluorene (AAF). DEN i.p. (200 mg·kg^{-1} b.w.) as initiator. After a 2-week recovery period, the rats were then fed a diet containing 0.02% AAF for 2 months as a promoter.	A γ-tocotrienol-enriched fraction (consisting of 80% γ-tocotrienol and 20% α- and β-tocotrienol) was used. Tocotrienol was given at a dose of 30 mg·kg^{-1} body weight in the diet. Tocotrienol supplementation coincided with the AAF feeding period and continued until sacrifice.	• Rats given AAF + tocotrienol → ↓off-white liver patches & ↓ultrastructural damage. Significantly ↓plasma GGT (12 and 20 weeks) & ↓liver microsomal UDP-GT activity (20 weeks). • Rats given DEN/AAF + tocotrienol → ↓ neoplastic nodule. ↓cellular damage. More normal liver morphology. ↓plasma GGT, ↓liver GSSG-RX, and ↓GST activities (significant). ↓liver GGT & ↓GSH-Px activities (not significant).	Tocotrienols protect against chemically induced liver injury in rats by reducing neoplastic transformation, restoring glutathione-dependent enzymes and GGT toward control levels, modulating detoxification pathways, and preserving liver structure, thus reducing liver damage and preneoplastic changes, as shown by ultrastructural, morphological, and histological evidence.
Iqbal *et al*. (2004) [72]	Male Sprague–Dawley rats. Liver injury model → diethylnitrosamine (DEN)/2-acetylaminofluorene (AAF)-induced hepatocarcinogenesis in rats. Single dose of DEN i.p. (200 mg·kg^{-1} b.w.) After DEN injection → rats given diet containing 0.02% (w/w) AAF for 2 months.	T3-Rich Fraction (TRF) isolated from refined edible grade rice bran oil (RBO). (10 mg·kg^{-1} b.w./day by gastric intubation).	• Rats given DEN/AAF + TRF → Significant ↓ in off-white liver patches & small liver nodules. ↓ rise of plasma alkaline phosphatase (ALP) activity by 29%. ↑ hepatic ALP by additional 48% → indicates ↑hepatic detoxification. ↓ rise in hepatic glutathione S-transferase (GST) by 37% → suggesting ↓ carcinogen-induced toxicity. ↓ the rise in lipid peroxidation (TBARS) by 66%. ↓ rise of LDL oxidation by 62%.	TRF protects against hepatocarcinogenesis and liver injury by reducing lipid and protein oxidation through its antioxidant properties. Long-term supplementation lowers hepatic oxidative stress and carcinogen-induced damage, offering significant protection against liver cancer development.

Table 1. Continued.

Study (year)	Animals (cells) used & liver injury model	Tocotrienol form & dose	Main outcomes	Conclusion
Yachi *et al*. (2010) [73]	Male SD-IGS rats. Liver injury model → Carbon tetrachloride (CCl4) (0.5 mL/kg b.w.) administered orally (6 hours after the rats received the vitamin E analogs).	γ-tocopherol (γ-Toc) and a tocotrienol mix (T3), with α-tocopherol (α-Toc) included as a reference. The T3 mix contained α-T3 (37.8%), β-T3 (4.0%), γ-T3 (45.5%), and σ-T3 (10.7%). Each rat received 20 mg of the respective vitamin E compound as a single pre-treatment dose (6 hours before liver injury induction by CCl_4).	• CCl_4 treatment → ↑liver enlargement + ↑triglyceride (TG) levels, both significantly ↓↓↓ by T3 co-treatment. • CCl_4 + T3 co-treatment → ↓liver lipid buildup T3 conteracts ↓metabolic enzymes. → histological analysis → ↓lipid accumulation compared to CCl_4 alone. • All groups → total liver cholesterol levels remained similar. • T3 → ↓ALT activity (indicating less liver cell damage), ↑↑↑ antioxidant effects than α-tocopherol. • CCl_4 → increased inflammatory cytokine levels (vitamin E analogs unable to ↓ this effect).	Vitamin E analogs improve CCl_4-induced liver damage in rats → T3s > γ-Toc. Reports of tocotrienols (T3s) being effective in non-alcoholic steatohepatitis (NASH) suggest similar therapeutic potential for γ-tocopherol (γ-Toc) and T3s in related conditions such as NAFLD/NASH. Both compounds may serve as promising treatments, but further studies are needed to compare individual T3 analogs and validate results across experimental models.
Yachi *et al*. (2013) [76]	Male SD-IGS rats. A rat model of steatohepatitis was developed by feeding rats a high-fat diet followed by D-galactosamine (GalN) and tumor necrosis factor-α (TNF-α) administration to induce inflammation and mimic Non-alcoholic steatohepatitis (NASH) pathology. *In vitro* model of steatohepatitis. Primary hepatocytes from rats on vitamin E-deficient diets were stimulated with TNF-α.	In the *in vivo* study, a tocotrienol mixture containing 37.8% α-T3, 4.0% β-T3, 45.5% γ-T3, and 10.7% σ-T3 (98% purity) was incorporated into experimental diets. Rats were fed either a T3-enriched high-fat diet (800 mg·kg^{-1}) or a combined αToc and T3-enriched diet (800 mg T3 + 50 mg α-Toc/kg) for 4 weeks. In the *in vitro* study, α-T3 and γ-T3 were used, with general T3 added to the hepatocyte culture medium at a concentration of 1×10^{-9} M during initial seeding. While α-T3 and γ-T3 were later added to assess their effects on inflammatory cytokine mRNA expression, their exact concentrations during this phase were not specified.	• Combination of αToc + T3 → significantly ↓ liver triglyceride accumulation, ↓lipid peroxidation (TBARS), ↓liver damage markers (AST, ALT), ↓inflammatory cytokine (IL1-β, IL6), ↓TGF-β1 mRNA expression ↓decreased liver fibrosis. These effects → ↑↑↑ in (αToc + T3) group → synergistic action → ↑antioxidative capacity + ↓liver inflammation & fibrosis. • *In vitro* study (rat primary hepatocytes) → TNF-α stimulation ↑IL1-β & ↑IL6 mRNA expression → significantly ↓↓↓ by α-Toc/γ-T3. • γ-T3 → ↓TGF-β1 mRNA expression & ↓Smad3 mRNA levels → potential role in ↓inflammatory & ↓fibrotic signaling pathways.	Tocotrienol intake inhibits lipid accumulation, inflammation, and fibrosis in the liver in this rat model of steatohepatitis, and these effects are reinforced synergistically by simultaneous intake of α-tocopherol and tocotrienol.

Table 1. Continued.

Study (year)	Animals (cells) used & liver injury model	Tocotrienol form & dose	Main outcomes	Conclusion
Lee *et al*. (2010) [74]	Male Wistar rats. Hepatotoxicity was induced by CCl_4 administration.	TRF from palm oil (oral gavage). 25 mg·kg^{-1} b.w. & 50 mg·kg^{-1} b.w. TRF → administered once daily (8 weeks).	• TRF treatment ↓↓↓ sGOT (68–73%) & ↓↓↓ sGPT (47–83%) levels → indicating improved liver function. • TRF → ↑hepatoprotective properties → significant alterations in various oxidative stress parameters within the liver & ↑antioxidant enzyme activity.	TRF protected rats against CCl_4-induced liver injury by lowering liver enzyme levels (sGOT, sGPT), reducing oxidative stress markers, and enhancing antioxidant enzyme activity. Its effects were comparable to silymarin, suggesting TRF protects the liver by limiting oxidative damage and strengthening antioxidant defences.
Ayu Jayusman *et al*. (2014) [77]	Male Sprague-Dawley rats. Liver injury induction → oral administration of fenitrothion (FNT), (20 mg·kg^{-1} for 28 days). FNT causes liver damage → generating excessive ROS → oxidative stress → membrane damage in tissues.	Palm oil TRF (200 mg·kg^{-1} for 28 days). TRF → given 30 minutes before FNT administration.	• TRF ↑ liver biochemical markers in FNT-treated rats. • TRF ↓ FNT-induced liver morphological changes	TRF supplementation protects liver from FNT-induced damage. TRF improves biochemical and morphological liver changes.
Abdella *et al*. (2014) [79]	Adult males laboratory mice (*M. musculus*). The liver injury model → valproic acid (VPA) (100 mg·kg^{-1}/day i.p. for three weeks).	Vitamin E (50 mg·kg^{-1}, 100 mg·kg^{-1}, and 200 mg·kg^{-1}). Vitamin E was administration → oral intubation once daily. Vitamin E treatment (alone or in concomitant with VPA) → daily for three weeks.	• Vitamin E (50 & 100 mg·kg^{-1}) treatments → ↓↓↓VPA-induced hepatic histopathological lesions. • VPA + vitamin E (100 mg·kg^{-1}) → normal structure of mitochondria, nucleus, cell membrane & normal glycogen distribution. • VPA + 200 mg·kg^{-1} vitamin E → liver tissues still showed dilated portal veins, inflammatory cell infiltration & hepatic cells mild hydropic degeneration.	Vitamin E at 100 mg·kg^{-1} showed the strongest ameliorative effect, while both 50 mg·kg^{-1} and 100 mg·kg^{-1} effectively decreased chromosomal aberrations, increased the mitotic index and reduced hepatopathological lesions. Higher (200 mg·kg^{-1}) was less effective.
Kamisah *et al*. (2014) [80]	Male Wistar rats. The liver injury model → administration of phenylhydrazine.	Palm oil TRF (30 mg·kg^{-1}/day given i.p for 14 days)	• TRF Pretreatment → ↓oxidative stress caused by phenylhydrazine (↓TBARS in erythrocytes and liver tissue). • TRF + phenylhydrazine → ↓liver heme oxygenase & ↓biliverdin reductase activities. • ↓biliverdin reductase activity → may be due to lower biliverdin levels → resulting from ↓ heme oxygenase activity & ↓overall oxidative stress.	TRF significantly ↓ phenylhydrazine-induced hyperbilirubinemia, most likely through its antioxidant effects and inhibition of hepatic heme oxygenase and biliverdin reductase activities.

Table 1. Continued.

Study (year)	Animals (cells) used & liver injury model	Tocotrienol form & dose	Main outcomes	Conclusion
Tan *et al*. (2015) [12]	Liver injury model → immortalised murine transforming growth factor alpha (TGF-α) transgenic hepatocyte (TAMH) cells treated with paracetamol (APAP) and hydrogen peroxide (H_2O_2).	α-Toc and T3 analogs (α-T3, σ-T3 & γ-T3) were used in this study. α-TP & α-T3 were not toxic up to 100 mM, while σ-T3 & γ-T3 showed toxicity at 10 mM and above. For protective effect evaluations against toxicants, concentrations of 10 & 50 mM were used for α-TP & α-T3, while 5 & 10 mM were used for σ-T3 & γ-T3. The analogs were applied using two distinct *in vitro* treatment approaches, both for 24 hours.	• α-T3 → ↑hepatoprotection than α-TP against liver injury. Both α-T3 & α-TP → ↓oxidative stress & ↓apoptosis. • α-T3 → ↑potency & ↑cellular uptake for hepatoprotection. • Tocotrienol (particularly α-T3) → ↑hepatoprotective effects against drug-induced liver injury → ↓oxidative stress, ↓reactive oxygen species & ↑liver regeneration.	αTP/αT3 exhibit protective effects against liver injury induced by APAP & H_2O_2 in a dose dependent manner. These effects are achieved by neutralizing free radicals, alleviating mitochondrial stress, inhibiting oxidative stress and promoting body's natural anti-oxidative defense.
Kamisah *et al*. (2015) [81]	Male Wistar rats. The liver injury model used was a high-methionine diet (1% methionine) given to rats for 10 weeks, which caused liver injury by significantly increasing plasma homocysteine levels and hepatic oxidative stress.	Palm oil TRF. TRF (mixed in diet). 30 mg·kg^{-1} diet (low dose), 60 mg·kg^{-1} diet (moderate dose), and 150 mg·kg^{-1} diet (high dose). The diets were given from week 6 to week 10 of the study. Throughout the entire 10-week period, all rats were also fed a high-methionine diet containing 1% methionine.	• High-methionine diet → ↑Hepatic lipid peroxidation. • TRF supplementation → ↓hepatic lipid peroxidation content (measured as TBARS) → ↓oxidative stress in the liver. • TRF → ↓liver cystathionine β-synthase activity (which controls the rate of the transsulphuration pathway) due to high-methionine diet.	TRF → ↓liver injury in methionine-treated rats by ↓hyperhomocysteinaemia & ↓hepatic oxidative stress, (potentially through inhibiting cystathionine β-synthase).
Mostafa & Adel (2016) [75]	Male Sprague Dawley rats. Liver injury → CCl_4 0.5 mL/kg i.p. (2×per week for six weeks).	T3 (60 mg·kg^{-1}). T3 control group → 60 mg·kg^{-1}/day for 6 weeks. Treatment group → CCl_4 injections 2X weekly and daily T3 for 6 weeks. Protection group → T3 daily for 2 months before CCl_4 treatment, then CCl_4 injections 2X/week and T3 daily for 6 weeks.	• T3 supplementation → ↑liver function & ↓CCl_4-induced liver damage, ↓steatosis, ↓necrosis, ↓fibrosis, ↓oxidative stress markers, ↑antioxidant enzyme activity, ↓levels of profibrotic growth factors (PDGF & TGF-β).	TE supplementation can have beneficial effects on liver functions and an effective role for the prevention of CCl_4 induced hepatic damage in rats.
Ayu Jayusman *et al*. (2017) [78]	Male Sprague-Dawley rats. Liver injury induction → oral administration of fenitrothion (FNT), (20 mg·kg^{-1} for 28 days).	Palm oil TRF. (200 mg·kg^{-1} b.w. 28 days p.o.). TRF → 30 minutes before FNT administration.	• TRF ↓ oxidative liver damage from fenitrothion. • TRF ↑antioxidant status & ↑liver enzyme levels.	TRF protects against FNT-induced liver damage. Further studies needed to explore underlying mechanisms and recovery levels.

Footnote: ↑ increased, ↓ decreased, → leads to.

increased γ-glutamyl transpeptidase (GGT) and UDP-glucuronyltransferase (UDP-GT) activity and caused cellular changes indicative of early liver neoplasia. Rats receiving T3s alongside AAF exhibited significantly reduced enzyme activity and tissue damage [71]. A subsequent long-term study investigated hepatic carcinogenesis induced by AAF alone or combined with Diethylnitrosamine (DEN). Male rats fed a TRF exhibited increased activities of detoxification enzymes [gamma-glutamyltransferase (GGT), uridine diphosphate glucuronosyltransferase (UDP-GT), glutathione reductase (GSSG-RX), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST)] and a reduction in preneoplastic lesions compared to controls [70]. Together, these pioneering studies provided the first evidence that T3s enhance detoxification capacity, protect against carcinogen-induced liver injury, and hold therapeutic potential in cancer prevention.

The effect of TRF from rice bran oil in a rat model of liver cancer induced by DEN and AAF had been evaluated [72]. Carcinogen exposure caused marked liver alterations and changes in alkaline phosphatase (ALP) and glutathione S-transferase (GST), sensitive markers of preneoplastic and neoplastic progression. The study linked these effects to oxidative stress, highlighting LPO and malondialdehyde (MDA) as drivers of carcinogenesis. Notably, TRF significantly reduced tumour development, with protection attributed to its antioxidant activity in lowering LPO and protein oxidation. These findings suggest TRF mitigates oxidative stress-mediated hepatocarcinogenesis [72].

T3s protect against chronic carbon tetrachloride (CCl_4)-induced liver injury by reducing oxidative stress, lowering plasma ALT, and decreasing the incidence of fatty liver. They also limit Triglyceride (TG) accumulation and liver enlargement, likely through effects on lipid metabolism and enzyme activity. α-T3 appears central to this action. Although plasma levels decline, hepatic α-T3 remains stable, suggesting prolonged retention under CCl_4 exposure and enhanced radical scavenging. Furthermore, T3 treatment alleviates CCl_4-induced histopathological changes and improves liver function, as indicated by serum markers. These findings suggest that T3 has significant hepatoprotective potential and may serve as a promising therapeutic agent against CCl_4-induced liver injury [73–75].

T3 treatment in a TNF-α/D-galactosamine-induced rat model of steatohepatitis reduced liver TGs, LPO, and damage markers while suppressing inflammatory cytokine expression, leading to less inflammation and fibrosis. *In vitro*, α- and γ-T3 inhibited TNF-α-driven cytokines, with γ-T3 lowering TGF-β1. These findings highlight T3s' hepatoprotective benefits in steatohepatitis [76].

Fenitrothion (FNT) administration (20 mg·kg^{-1}, 28 days) caused marked hepatotoxicity. This leads to significant declines in body and liver weights, elevated serum liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT)], total protein, and bilirubin, as well as oxidative stress evidenced by increased MDA and protein carbonyl (PCO), reduced antioxidants [glutathione (GSH), ferric reducing antioxidant power (FRAP), glutathione peroxidase (GPx)], and histological injury including haemorrhage, necrosis, mitochondrial swelling, and disrupted nuclear membranes. Co-treatment with TRF (200 mg·kg^{-1}) significantly alleviated these effects by improving weights, normalizing ALT and AST levels, reducing MDA and PCO, restoring antioxidant levels, and preserving hepatic architecture. However, TRF presented no significant effect on GGT, bilirubin, or cholinesterase. Nevertheless, overall findings support its antioxidant-mediated cytoprotective role against FNT-induced hepatotoxicity [77,78].

Vitamin E demonstrates protective effects against valproic acid (VPA)-induced chromosomal changes in mice. The mice were divided into control, VPA-treated, and vitamin E groups. Animals received oral doses of 50, 100, or 200 mg·kg^{-1} vitamin E by intubation or gavage. Accordingly, vitamin E at 50 and 100 mg·kg^{-1} significantly reduced VPA-induced chromosomal alterations, which are associated with hepatotoxicity. These results suggest that vitamin E may serve as a therapeutic agent for mitigating VPA-related liver damage. Nonetheless, further research is needed to clarify the underlying mechanisms and establish optimal dosing strategies for effective hepatoprotection [79].

TRF from palm oil demonstrates hepatoprotective potential in a phenylhydrazine-induced liver injury model. In male Wistar rats, TRF (30 mg·kg^{-1}, intraperitoneal) significantly reduced phenylhydrazine-induced hyperbilirubinemia. This protective effect is attributed to its antioxidant activity and the inhibition of hepatic heme oxygenase and biliverdin reductase, highlighting TRF's role in mitigating oxidative liver injury [80]. Furthermore, a high-methionine diet induces hepatic stress and potential liver injury by promoting oxidative stress and disrupting methylation pathways. In rats, TRF supplementation reduced oxidative stress, lowered hyperhomocysteinaemia, and may directly inhibit hepatic cystathionine β-synthase. Compared to controls, TRF administration improved outcomes in high-methionine diet groups. As such, these findings suggest that TRF protects against methionine-induced liver injury, primarily through its antioxidant properties and modulation of homocysteine metabolism [81].

8. Clinical Evidence: Tocotrienol in Liver Health and Implications for DILI

Emerging clinical research has started to investigate the potential benefits of T3s on human liver function, particularly in relation to NAFLD. In individuals diagnosed with NAFLD, supplementation with δ-T3 has been linked to improvements in several liver-related biomarkers. This includes reduced levels of serum aminotransferases (ALT

Table 2. Summary of clinical studies on tocotrienols in liver health (with implications for DILI).

Authors	Study design	Population	Tocotrienol isoform & dosage	Duration	Key findings	Conclusion
Pervez *et al*. 2018 [3]	Randomized, double-blind, placebo-controlled	NAFLD patients	δ-T3 600 mg/day	12 weeks	Decreased serum aminotransferases (ALT: –13.0; AST: –8.77), hs-CRP (–0.74), MDA (–0.89), FLI score (–9.41). No improvement in hepatic steatosis on ultrasound.	δ-T3 → reduced liver enzymes, inflammation, and oxidative stress in NAFLD patients.
Magosso *et al*. 2013 [82]	Randomized, double-blind, placebo-controlled	Hypercholesterolemic adults with NAFLD	Mixed T3 400 mg/day	1 year	A significantly higher normalisation of hepatic echogenic response in the tocotrienols-treated group (50%) compared to the placebo group (23.5%).	Mixed T3 → liver-protective effects of in hypercholesterolemic adults with NAFLD.
Thendiono 2018 [84]	Interventional comparative study design	NAFLD patients	Mixed Tocotrienol 100 mg/day plus lifestyle modification	3 months	Mixed tocotrienols significantly decreased liver stiffness measurement (LSM) among NAFLD patients (79%).	T3 → positive impact of on liver stiffness in the target population.
Pervez *et al*. 2020 [83]	Randomized, double-blind, placebo-controlled	NAFLD patients	δ-T3 600 mg/day	24 weeks	Reduced FLI (–12.82), HOMA-IR (–0.52), hs-CRP 9 (–0.6), MDA (–0.91), ALT (–8.86), AST (–6.6). Reduced hepatic steatosis.	δ-T3 → improved biochemical markers of hepatocellular injury and steatosis in NAFLD patients.
Nawawi *et al*. 2022 [85]	Interventional comparative study design	Patients with metabolic dysfunction-associated fatty liver disease (MAFLD)	Palm oil TRF 50 mg/day	6 months	Significant reduction in ALT (–37.4 U/L) and AST (–9.5 U/L) levels observed. Improvement in hepatic steatosis (+0.36) and inflammation (+0.19) scores noted.	TRF → strong potential for treating fatty liver disease, both with and without steatohepatitis.

and AST), high-sensitivity C-reactive protein (hs-CRP), MDA, and the fatty liver index (FLI) [3]. Evidence from a randomized, double-blind, placebo-controlled pilot trial supports the safety and efficacy of δ-T3 in reducing inflammation and oxidative stress in this population. Despite these biochemical improvements, the study did not report a notable reduction in liver fat accumulation when assessed by ultrasound imaging [3].

Another clinical trial investigated the effects of mixed palm T3s in hypercholesterolemic patients with ultrasound-proven NAFLD [82]. The results of this study indicated that treatment with mixed T3s led to a significant normalization of the hepatic echogenic response, a measure of fat content in the liver, compared to the placebo group [82]. This finding suggests a potential for T3s to directly impact the accumulation of fat in the liver. Furthermore, δ-T3 supplementation has been demonstrated to improve biochemical markers of hepatocellular injury and steatosis in patients with NAFLD in a randomized, placebo-controlled trial conducted over 24 weeks [83]. Studies have also indicated that TRF can improve liver stiffness, a marker of fibrosis, in patients with NAFLD [84]. A more recent clinical trial demonstrated that palm TRF supplementation significantly improve transaminase levels, hepatic steatosis, and inflammation scores in patients with metabolic dysfunction-associated fatty liver disease [85]. Considering that NAFLD shares common pathophysiological features with DILI, such as oxidative stress and inflammation, these positive findings in NAFLD patients suggest that T3s may also offer benefits in the context of DILI [49].

Although preclinical studies and clinical trials in NAFLD have demonstrated encouraging results, there remains a limited number of clinical investigations specifically examining T3s for the treatment of DILI. Current literature suggests potential hepatoprotective effects of α-T3 in the context of DILI. However, direct clinical evidence supporting this application is apparently lacking [12]. There is a lack of clinical data that directly focuses on this topic, leaving a critical gap in current research. Although studies on NAFLD and preclinical models demonstrated great potential, direct evidence from clinical trials is needed to prove the efficacy of T3s in patients with DILI. A summary of clinical studies on T3s in liver health (with implications for DILI) is summarized in Table 2 (Ref. [3,82–85]).

Nevertheless, there are significant key differences between DILI and NAFLD that cannot be ignored. In NAFLD, metabolic stress is central: chronic nutrient excess and insulin resistance drive hepatic lipotoxicity. This leads to ER stress, mitochondrial dysfunction, oxidative stress, and sterile inflammation, consistent with the "multiple parallel hits" model that incorporates adipose lipid flux and gut-liver interactions [86–88]. By contrast, DILI is usually initiated by xenobiotics. Drugs or their metabolites can induce injury through reactive metabolite formation, mitochondrial or ER stress, or bile acid dysregulation, such as BSEP inhibition. Meanwhile, in iDILI, adaptive immune responses (modulated by HLA risk alleles) play a critical role, consistent with the "hapten" and "danger" models [2,45,46]. Despite distinct triggers, both NAFLD and DILI converge on oxidative/ER stress, mitochondrial dysfunction, and inflammatory signalling. The significant difference is that NAFLD reflects chronic metabolic overload, whereas DILI arises from a specific xenobiotic exposure. Moreover, iDILI involves an abrupt, antigen/HLA-restricted immune component. Conversely, NAFLD inflammation is metabolically programmed, whereas iDILI immunity is host- and antigen-specific [45,87]. There is a mechanistic link between NAFLD and several forms of DILI that justifies using T3s as a potential therapeutic treatment. However, there are apparent exceptions, particularly for immune-mediated iDILI and DILI caused by transporter-blocked cholestasis. Extrapolation is justified where oxidative/ER stress, mitochondrial injury, inflammasome activation, and miRNA signatures dominate, particularly in intrinsic DILI and drug-induced steatohepatitis. Still, it is not warranted for HLA-restricted iDILI (immune-driven) or BSEP-block cholestasis (transporter-related), since T3s are unlikely to address the underlying cause. This argues for adjunctive (not replacement) use of T3 in carefully phenotyped DILI, supported by mechanistic biomarkers to confirm target engagement.

T3s have demonstrated encouraging effects in advanced liver disease, particularly in slowing progression toward cirrhosis. Although research remains at an early stage, emerging studies highlight potential mechanisms and clinical benefits. The most notable investigation examined T3 supplementation in patients with End-Stage Liver Disease (ESLD) awaiting transplantation [89]. Using the Model for End-Stage Liver Disease (MELD) score, a standard tool for assessing chronic liver disease severity and transplant priority, the study revealed that oral T3 supplementation reduced MELD scores in 50% of treated patients. This is compared with only 20% in those given α-tocopherol, the common form of vitamin E. Since a lower MELD score reflects improved liver function and a reduced mortality risk, these results suggest that T3s may help stabilize liver disease in critically ill patients and potentially delay the need for transplantation [89]. Nonetheless, no large, randomized trials have yet demonstrated improvements in survival, decompensation, transplant-free survival, or quality of life in ESLD. Therefore, the current evidence should be considered preliminary and hypothesis-generating. In line with this, T3s may benefit ESLD by reducing oxidative stress, suppressing inflammation, limiting fibrosis through effects on HSCs, and improving lipid metabolism, addressing key drivers of liver injury and progression [40,90].

Table 3. Postulated key hepatoprotective mechanisms of tocotrienols relevant to DILI.

Mechanism	Supporting Evidence	References
Scavenging Reactive Oxygen Species and Reducing Oxidative Stress	Potent antioxidant activity, inhibits free radical production and lipid peroxidation, α-T3 more active than α-Toc in rat liver microsomes.	Tan *et al*. 2015 [12]
Modulating Inflammatory Pathways	Possesses anti-inflammatory properties, inhibits expression of TNF-α, IL-1, IL-6.	Yachi *et al*. 2013 [76]
Potential for Enhancing Cellular Defense Mechanisms (e.g., Nrf2 Activation)	TRF activates Nrf2 pathway in mouse liver, leading to increased expression of antioxidant and cytoprotective genes.	Atia *et al*. 2021 [34]
Impact on Lipid Metabolism and Cellular Membrane Integrity	Influences lipid metabolism, reduces triacylglycerol levels in hepatocytes, efficiently penetrates and distributes within cell membranes.	Pervez *et al*. 2018 [3]
Regulation of MicroRNA Expression	δ-T3 and α-Toc downregulate miRNAs involved in steatosis, insulin resistance, oxidative stress, inflammation, and apoptosis in NAFLD patients.	Pervez *et al*. 2022 [44]

9. Challenges, Limitations, and Future Directions

Based on the preclinical and clinical studies discussed above, it could be summarized that T3s help protect the liver from drug-induced damage mainly through their strong antioxidant properties. Specifically, they lower oxidative stress, reduce the formation of ROS and LPO, and increase the activity of natural antioxidant defenses such as reduced glutathione, SOD, catalase, and GPx. In addition, T3s help normalize elevated liver enzymes, including ALT, AST, and GGT, and improve liver tissue viability by reducing fat accumulation (steatosis), cell death (necrosis), and structural damage. They also suppress inflammation by decreasing the production of pro-inflammatory cytokines such as TNF-α and IL-6. In some cases, T3s may also help by preventing the activation of HSCs and supporting better fat metabolism in liver cells. The plausible key mechanisms in which T3s protect against DILI are listed in Table 3 (Ref. [3,12,34,44,76]) and Fig. 2.

Current research has identified several crucial gaps that need to be considered when examining how T3s may help treat DILI. One major issue is that most studies have focused on how T3s work in NAFLD, not in DILI specifically. Although both NAFLD and DILI involve factors such as inflammation and oxidative stress, their underlying causes and patterns of disease progression differ significantly [40]. Due to these aspects, results from NAFLD studies cannot be directly applied to DILI, and additional research is required to understand how T3s affect drug-related liver damage.

One notable limitation is the low bioavailability of T3s, which may reduce their effectiveness when taken orally [25]. This issue suggests the need to investigate alternative formulations or delivery methods that could improve absorption and increase their concentration in target tissues [91]. In addition, existing studies vary widely in terms of T3 dosage and treatment duration, making it challenging to determine the most effective approach for managing liver injury [49]. Moreover, large-scale randomized controlled trials are still limited, especially those that examine liver injury other than NAFLD. The diversity in study populations and the types of DILI studied further complicates efforts to draw clear conclusions from current evidence.

In intrinsic DILI, T3s protect the liver mainly by reducing oxidative/ER stress and inflammation. They lower mitochondrial ROS, enhance Nrf2-dependent defenses (HO-1, NQO1), and suppress NF-κB/NLRP3 signaling, leading to improved injury markers. These mechanisms are particularly relevant in toxicant-induced DILI (e.g., acetaminophen, isoniazid), where oxidative stress is the primary driver.

In iDILI, T3s may protect by controlling oxidative stress, dampening TLR4–NF-κB and NLRP3 signaling, and reducing ER-mitochondrial stress, acting upstream of T-cell-mediated injury. While emerging targets such as PD-L1 signaling and miRNA pathways are intriguing, they remain unassessed in iDILI. Furthermore, key gaps include the lack of human data, transporter effects, and validation in HLA-risk models. Hence, targeted studies in immune-competent and cholestatic systems are needed to define their therapeutic potential.

Future research on T3s in DILI should prioritize well-designed clinical trials that assess the effects of specific isoforms across different types of DILI. Determining optimal dosage, duration, and formulations that enhance bioavailability will be crucial, as will evaluating their potential synergy with current DILI treatments. Studies should also investigate the molecular pathways underlying their hepatoprotective actions, focusing on isoform-specific effects. Additionally, assessing the preventive potential of T3s in high-risk individuals could expand their clinical relevance. Ultimately, targeted clinical trials are needed to validate preclinical findings and insights gained from related conditions such as NAFLD.

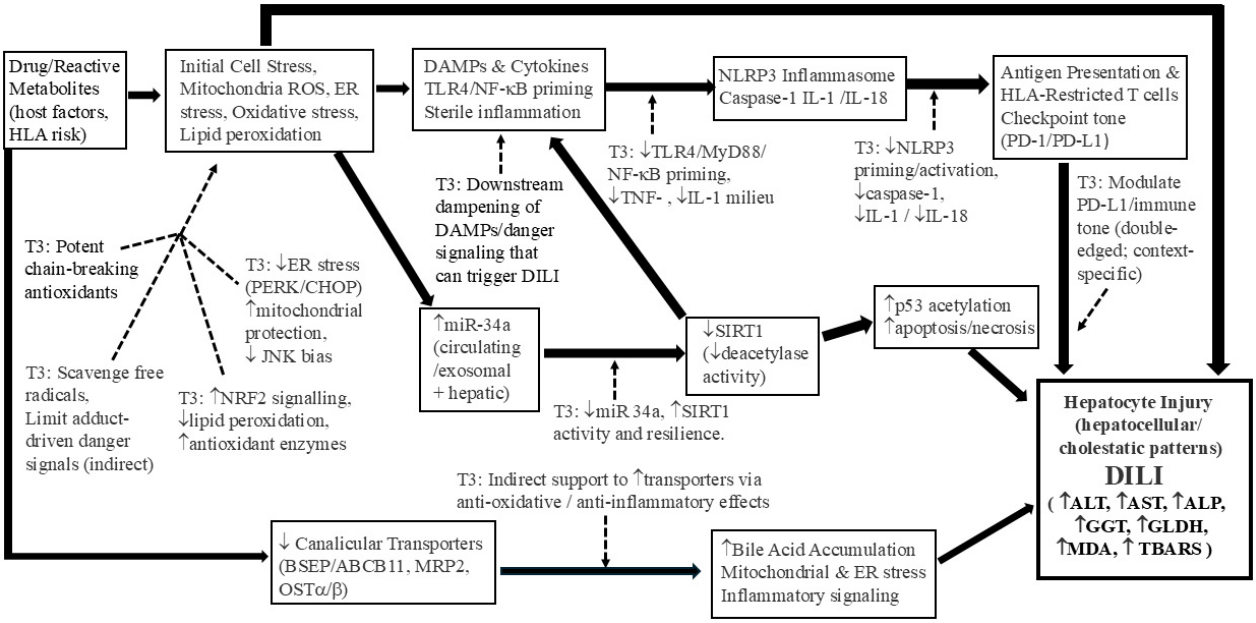


Fig. 2. Tocotrienol-mediated mechanisms for the prevention and limitation of drug-induced liver injury (DILI). The filled arrows represent the mechanistic pathways involved in DILI, in which the thicker size of the arrows represent the more important mechanistic pathways in DILI pathogenesis. The broken arrows represent the effect of tocotrienols in mitigating DILI at the various mechanistic steps. ↑ increased, ↓ decreased.

10. Conclusion

T3s show promise as therapeutic agents in DILI by addressing major pathogenic mechanisms. Their strong antioxidant properties neutralize ROS and reduce LPO, protecting hepatocytes from reactive drug metabolites. They may also preserve mitochondrial integrity, vital for cell survival under toxic stress, and exert immunomodulatory effects that could benefit immune-mediated DILI. Additionally, evidence from clinical studies in NAFLD suggests T3s protect the liver in humans, supporting their potential relevance for DILI. Despite these encouraging findings, a shortage of well-designed clinical trials specifically focused on evaluating the role of T3s in DILI remains. Therefore, addressing this gap should be a priority in future research to establish the therapeutic value of these approaches in this context.

Author Contributions

AA conceived and initiated the review, designed the study framework, and supervised the project. NAMF provided guidance, critical advice, and contributed to the conceptual development of the manuscript. NSSMN, MRAR, JAH, and FZJS conducted literature searches, drafted sections of the manuscript, and assisted with data compilation and synthesis. AA and NAMF critically revised the manuscript for intellectual content. All authors contributed to editorial changes, read, and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

While preparing this manuscript, the authors utilized ChatGPT-4 to help identify spelling and grammatical issues. Following this, all content was carefully reviewed

and edited by the authors, who assume full responsibility for the final version of the manuscript.

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