

REVIEW ARTICLE

Hepatotoxicity of titanium dioxide nanoparticles

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Abstract

The food additive E171 (titanium dioxide, TiO₂), is widely used in foods, pharmaceuticals and cosmetics. It is a fine white powder, with at least one third of its particles sized in the nanoparticulate (<100 nm range, TiO₂ NPs). The use of E171 is controversial as its relevant risk assessment has never been satisfactorily accomplished. In vitro and in vivo studies have shown dose-dependent toxicity in various organs including the liver. TiO₂ NPs have been shown to induce inflammation, cell death and structural and functional changes within the liver. The toxicity of TiO₂ NPs in experimental models varies between organs and according to their physiochemical characteristics and parameters such as dosage and route of administration. Among these factors, ingestion is the most significant exposure route, and the liver is a key target organ. The aim of this review is to highlight the reported adverse effects of orally administered TiO₂ NPs on the liver and to discuss the controversial state of its toxicity.

KEYWORDS

E171, liver, nanoparticles, oxidative stress, titanium dioxide

1 | INTRODUCTION

Nanotechnology has extensive applications in health and medicine, agriculture, environmental protection and the food industry. In the food industry, nanomaterials are used as coatings in packaging to protect from mechanical damage as well as to minimise microbial contamination. Nanoparticles (NPs) are also added to food to enhance taste, impression, colour, quality and consistency (Kassebaum & Collaborators, 2016; Shen et al., 2017; Von Moos et al., 2017).

Ingested NPs can be absorbed from the digestive tract, may accumulate in different organs and can trigger potential health risks. This comprehensive review summarises all available studies focussing on TiO₂ NPs and their impact on the liver. The review includes studies reporting various outcomes including toxicity as well as instances of no or minimal effects on the liver. Finally, this review provides a thorough discussion on the evidence of TiO₂ NP-induced liver toxicity

and its underlying mechanisms from research conducted in rodents and in cultured liver cells.

2 | APPLICATIONS OF TITANIUM DIOXIDE NPs

The most common compound of titanium is TiO₂, which constitutes almost 95% of all titanium consumed. Global production of TiO₂ was 6.1 million tons in 2016 and was expected to reach 7.8 million tons by the end of 2022, while its global market is predicted to increase by 8.9% per annum until 2025 (La Maestra et al., 2021).

TiO₂ are inorganic materials and are among the most common NPs found in consumer products (Hong et al., 2017). TiO₂ has some unique properties in ease of availability, biocompatibility, high specific surface area, long-term photostability, anti-corrosive, strong oxidising

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properties and antibacterial properties and are believed to have low toxicity. Due to these characteristics, paints and coatings occupy the biggest market share of total global production of TiO₂, that is, 48%. However, other application sectors include plastics (19%), resin (10%), papermaking (8%), fibre (3%), rubber (2%) and others (medicine, food and cosmetics; 10%) (Chen et al., 2020; Kaewklin et al., 2018; Yin et al., 2013). TiO₂ is used in a variety of cosmetics as it acts as an effective sunscreen and provides protection against short-wave ultraviolet (UV) radiation. It serves as a clouding agent and is assimilated into dry beverages and tobacco wrappings. Additionally, it is used in the pharmaceutical industry as a component of tablets (Weir et al., 2012). TiO₂ is used as an orthopaedic implant biomaterial, particularly for the hip and knee joints, bone plates, dental implants and dental products including crowns, bridges and dentures (Jacobs et al., 1991; Jin et al., 2022; Patri et al., 2009; Sul, 2010).

3 | FOOD

In the food industry, TiO₂ is known as E171 in the European Union (EU) and INS 171 in the United States (Korotcenkov, 2020b) (for simplicity, this review adopts the EU nomenclature). E171 is manufactured by two main processes, namely, a sulfuric acid-based process that yields the predominant crystalline forms of titanium dioxide, that is, anatase, rutile or a mixture of both, or a chlorine-based process that yields rutile only (Gázquez et al., 2014). E171 is commonly used in food and other consumer products such as toothpaste, coatings, preserved fruits, chewing gum, coated candy, carbonated drinks, dairy products, tattoo ink, sauces, icings, beauty creams (day creams, foundations and lip balms) and dressings (Keller et al., 2013; Lim et al., 2018; Lomer et al., 2000; Ortiz & Alster, 2012; Peters et al., 2014; Shi et al., 2013; Weir et al., 2012). Both anatase and rutile have been authorised to be used as a food additive; however, characterisation of food samples from Europe and America have shown that the general population is primarily exposed to anatase (Bischoff et al., 2021; Chen et al., 2013; Dufey et al., 2017). In the recent report of the European Food Safety Authority (EFSA) in 2021, it has been shown that the daily dietary intake of E171 in European countries is 11.5 mg/kg bw in children as compared to 6.7 mg/kg bw in adults. This intake does not include the amount of TiO₂ NPs coming from other non-food products like toothpastes, mouthwashes, medications and tattoo ink that can greatly impact to overall exposure (Fiordaliso et al., 2022; Younes et al., 2021; Zhang et al., 2018).

4 | ESTIMATED DIETARY INTAKE

Earlier studies have reported the estimated dietary intake in countries like the United States and the United Kingdom, where the oral consumption was around 1–2 mg/kg/day in children and 0.2–0.7 mg/kg/day in adults (Weir et al., 2012). It is clear that children are exposed to higher doses, with similar high exposure reported in British children (2.5–4.5 years), where it was above 3 mg/kg/day

(Lomer et al., 2000). Similarly, German children estimated consumption is reported to be around 2 mg/kg/day (Bachler et al., 2015), and Dutch children up to 2.16 mg/kg/day (Rompelberg et al., 2016) (details of all these studies are reviewed by Bischoff et al., 2021). The daily dietary intake of E171 is variable among different age groups and countries; however, due to lower body mass and disproportionately higher consumption of E171-containing products, children were found to be the most highly exposed group. Most children consume food more likely to contain TiO₂ such as candies, chewing gums and jellies (Bischoff et al., 2021; Li et al., 2018; Weir et al., 2012).

The maximum concentration of E171 as a food additive is 1% in the United States, while it was *quantum satis* levels (as much as needed, but not more) in the EU until it was banned in 2022 (Baranowska-Wójcik et al., 2022; Heringa et al., 2016; Rompelberg et al., 2016; Younes et al., 2021). The ideal properties that made it a preferred choice are its high abundance in nature, comparative cheapness, no nutritional value, insolubility in both water and organic solvents and presumed biological inertness (Buettner & Valentine, 2012; FDA, 2014; Korotcenkov, 2020a; Ropers et al., 2017; Zierden & Valentine, 2015).

5 | SAFETY

TiO₂ NPs were classed as potentially carcinogenic (by inhalation) by the International Agency for Research on Cancer (IARC, 2010). Through the course of time, several studies reported the adverse effects of TiO₂ NPs by various modes of administration (Hong et al., 2014; Hu et al., 2015; Ogunsuyi et al., 2022; Talamini et al., 2019). These reports led to the re-evaluation of the safety of TiO₂ as a food additive. The EFSA re-evaluated the usage of TiO₂ in 2016 and 2018 and concluded that oral intake of E171 has no concerns of genotoxicity and carcinogenicity (EFSA, 2016; Younes et al., 2018). However, in 2020, E171 usage was suspended in France for 1 year, and in 2021, it was declared unsafe to be used as a food additive (reviewed by Boutillier et al., 2021). Recently, the EU has also banned the usage of E171 as a food additive, as they have accepted that TiO₂ NPs may pose a risk of genotoxicity (Baranowska-Wójcik et al., 2022; Younes et al., 2021).

6 | ROUTE OF ENTRY, ABSORPTION AND ACCUMULATION OF TiO₂ NPs

Oral absorption of TiO₂ NPs is well studied in animal models (Jovanović, 2015); however, more investigations are needed in humans. Most ingested TiO₂ is not absorbed from the gut as several animal studies have confirmed that most of orally administered particles are excreted through the faeces (Cho et al., 2013; Farrell & Magnuson, 2017; Jo et al., 2016; MacNicoll et al., 2015). Orally administered TiO₂ NPs in rats are preferentially absorbed by Peyer's patches in the small intestine. Most of the consumed TiO₂ NPs are excreted via faeces, while the absorbed particles are translocated

across the gastrointestinal (GI) tract and enters the bloodstream (Kreyling et al., 2017; Riedle et al., 2020). It has been shown that TiO₂ NPs in mice accumulate mostly in the liver, spleen, kidneys, brain and lungs (Kreyling et al., 2017; Martins et al., 2017; Shinohara et al., 2014; Wang et al., 2007). Similarly, study in humans with a single oral dose of E171 demonstrated that >99% ingested particles were secreted in the stool and <1% were absorbed (Jones et al., 2015). Although TiO₂ NPs have shown poor absorption, human autopsies have shown that most accumulation occurs in the liver and spleen (Gilbert et al., 2021; Hamilton, 2013; Heringa et al., 2018; Keller et al., 1995; Lima et al., 2004; Peters et al., 2020; Younes et al., 2021) (see Figure 1).

TiO₂ NPs have been shown to induce toxicity in other organs including the small intestine (Acar et al., 2015; Coccini et al., 2015; Hu et al., 2011; Jia et al., 2017; Jugan et al., 2012; Orazizadeh et al., 2020; Petković et al., 2011; Salman et al., 2021; Valdiglesias et al., 2013), lungs (Fukatsu et al., 2018; Hussain et al., 2010; Koltermann-Jully et al., 2020; Li et al., 2010; Moon et al., 2010; Silva et al., 2013; Zhao et al., 2018), heart (Saber et al., 2013; Yu et al., 2014; Zhao et al., 2018), brain (Wu et al., 2008) (Heidari et al., 2019), kidneys (Hazelhoff et al., 2022) and spleen (Afshari-Kaveh et al., 2021). Several studies have reported the toxicity and adverse mechanisms of TiO₂ NPs in the liver via various modes of administration. Recently, an interesting postmortem study has shown

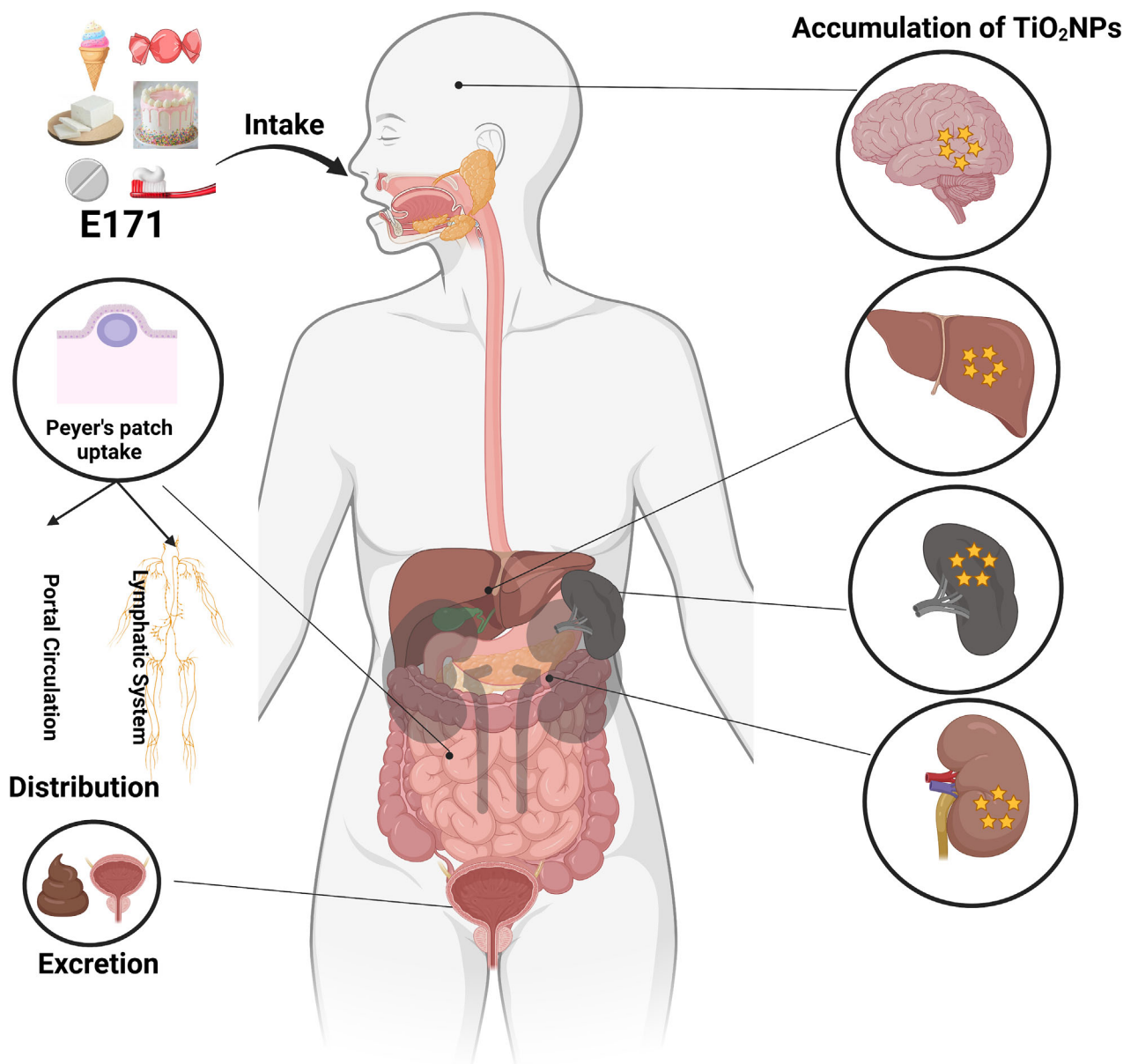


FIGURE 1 Route of entry, absorption, distribution, accumulation and excretion of TiO₂ nanoparticles (NPs). TiO₂ NPs enter the body through the oral cavity. Food elements and NPs get separated in the stomach and a small percentage of the particles are taken up by Peyer's patches in the small intestine from where they enter the bloodstream. Once distributed to the whole body, these particles accumulate in different organs including liver, brain, spleen and kidneys. However, the undistributed particles are excreted through faeces and urine. Created with BioRender.

the accumulation of TiO₂ NPs (24% of total TiO₂ particles were less than 100 nm) in the human liver (Heringa et al., 2018). All such results raise concerns about the cytotoxicity and liver impairment by the oral intake of food-grade TiO₂ NPs. This review focusses on the adverse effects after oral administration of TiO₂ NPs in the liver or studies demonstrating its effects in liver cells (in vitro). Databases including PubMed, Scopus and Web of Science were used with search terms “titanium dioxide OR titanium dioxide nanoparticle* OR nano titanium OR TiO₂ nanomaterial OR TiO₂ nanoparticle* OR TiO₂ food grade OR E171 AND liver OR hepatocyte* AND adverse effects OR toxicity”. Studies demonstrating other routes of administration like intraperitoneal (IP), intravenous (IV), inhalation or intratracheal instillation (IT) and subcutaneous (SC) administration or focusing other organs like lungs, intestine, kidneys, heart, brain and spleen were excluded. Studies used co-administration of TiO₂ NPs with any other substance/toxin/biomaterial or nanomaterial were also excluded. The flow diagram of the selection process is summarised in Figure 2.

7 | TiO₂ NPs AND LIVER TOXICITY

The liver is the most important organ for the detoxification of toxins and xenobiotics. Regardless of the route of administration, TiO₂ NPs

accumulate in the liver and can have toxic effects (Cui et al., 2010; Cui et al., 2011; Gilbert et al., 2021; Hassanein & El-Amir, 2018; Jia et al., 2017; Nie et al., 2021; Shirdare et al., 2022; Suker & Jasim, 2018; Wang et al., 2013). The reported mechanisms by which TiO₂ NPs may cause toxicity in the liver include generation of reactive oxygen species (ROS) (Jia et al., 2017; Shrivastava et al., 2014), oxidative stress (Chen et al., 2019; Chen et al., 2020; Wang, 2014), inflammation (Abbasi-Oshaghi et al., 2019; Hong et al., 2020), ultimately leading to steatosis, histopathological changes (fibrosis and damaged lobular structure) (Azim et al., 2015; Cui et al., 2011; Talamini et al., 2019; Tassinari et al., 2023; Wang et al., 2007; Wang et al., 2013; Zhao et al., 2021), DNA damage, necrosis, apoptosis and cell death (Cui et al., 2010; Jia et al., 2017; Orazizadeh et al., 2020).

This review focusses on studies showing toxicological effects of TiO₂ NPs. However, there are some studies that have reported very minor or no effects. These studies are shown in Table 1.

Overall, these studies report that oral administration of TiO₂ NPs result in their accumulation in the liver. TiO₂ NP accumulation leads to an increase in the levels of oxidative stress and inflammation, biochemical parameters, ultimately leading to histopathological changes, apoptosis, necrosis and decrease of antioxidants in the liver or its cultured cells.

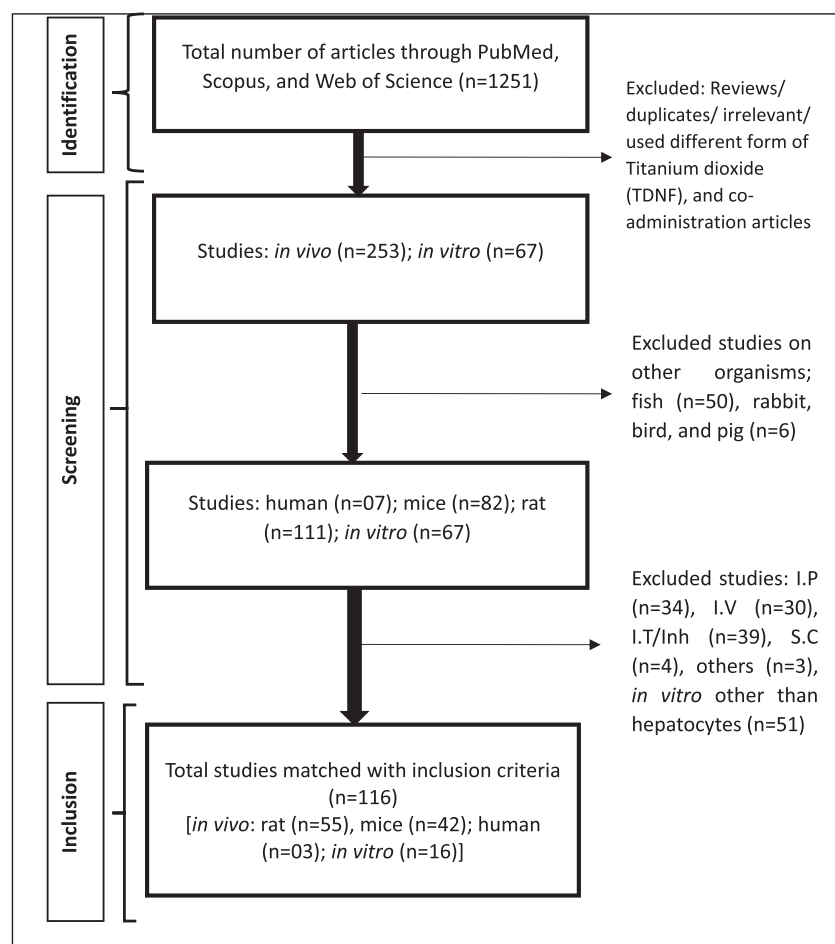


FIGURE 2 Flow chart of study selection process.

TABLE 1 Studies reported no or very minor effects of TiO₂ NPs on liver (in vivo).

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	General effects Effects on liver	References
Fischer 344 rats B6C3F1 mice (M, F)	25, 50	103 weeks	Anatase/NM	No change in body weight, but a few female mice died with in high-dose group No evidence of toxicity Non-significant signs of hepatocellular carcinoma White faeces in high-dose group	NCI (1979)
Sprague-Dawley rats (M, F)	100, 300, 1000, 5000	28 and 90 days	Rutile/rutile and anatase (A/R 79%–21%)/145 and 173 nm	No effects on mortality, body weights, organ weights and daily food consumption No change in neurobehavioral parameters, clinical chemistry, urine analysis or any clinical signs No gross or microscopic anatomic pathology or lesions Grey-coloured faeces in high-dose group	Warheit et al. (2015)
Sprague-Dawley rats (M, F)	500, 1000, 2000	Single dose	Anatase/rutile and mix/Uf 1, 2, 3 and Pg 1, 2, 3 Size range: 43, 42, 47, 153, 195, 213 nm	No increase of TiO ₂ in the blood and liver No increase in micronuclei and explicitly negative in the in vivo mammalian erythrocyte micronucleus test	Donner et al. (2016)
Wistar rats (M)	0.5	45 days/daily	Anatase/41.99 nm	No change in redox parameters No genotoxic effects No oxidative stress TiO ₂ accumulated in the liver	Martins et al. (2017)
Wistar Han IGS rats (M)	0.32, 32, 5	Daily/7 and 100 days	E171	No increase in liver to body weight No increase in inflammatory cytokines in plasma, colon or small intestine No histopathological conditions observed in the liver or any other organs No signs of carcinogenicity	Blevins et al. (2019)
Sprague-Dawley rats (F)	100, 300, 1000	Daily/14 days	A/R 80%/20%/21 nm	No mortality and change in body weight. No significant decrease in food consumption. No change in absolute and relative organ weights including liver, gravid uterine weight No change in caesarean section parameters, foetal weight, placental weight and placental macroscopic observation Titanium concentrations were elevated in maternal liver, maternal brain and placenta at high dose	Lee et al. (2019)
Sprague-Dawley rats (M, F)	250, 500, 1000	Daily/28 or 90 days	A/R 80%–20%/14–21 nm	No mortality, no gain or loss in body weight and food consumption. No ocular abnormalities or abnormal clinical signs	Heo et al. (2020)

(Continues)

TABLE 1 (Continued)

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	General effects Effects on liver	References
				No real differences in haematological, biochemical or urinalysis parameters There were no abnormal or gross findings in any of the animals at necropsy including the liver	
F344 rats (M, F)	10, 100, 300, 1000	Daily/28 or 90 days	Anatase/6 nm	No mortality and change body weight or even organ weight No change in parameters of urinalysis, haematology, serum biochemistry No genotoxicity (DNA strand breaks and chromosomal aberrations) in the liver No abnormality of colonic crypts TiO ₂ particles accumulated in the liver, kidneys and spleen	Akagi et al. (2023)
Sprague-Dawley rats (M, F)	10, 100, 1000	Daily/90 days	Anatase/40 nm	No mortality or abnormal clinical sign in eyes. No significant change in body weight or food consumption and no systemic toxicity No changes in parameters of haematology, urinalysis, clinical chemistry No abnormal gross findings at necropsy and no significant differences in endocrine-sensitive endpoints except for the absolute pituitary weights No Ti distribution in major tissues/organs No impact on microbiota diversity only a shift of community structure at genus level	Lin et al. (2023)

8 | BIOCHEMICAL PARAMETERS AND HISTOPATHOLOGICAL CHANGES IN THE LIVER

TiO₂ NPs increase the serum levels of liver injury markers including ALT, AST, LDH and ALP (Abbasi-Oshaghi et al., 2019; Azim et al., 2015; Niu et al., 2017; Sallam, Ahmed, Diab, et al., 2022). These biochemical indicators of injury are echoed by various histopathological liver changes including focal degeneration of hepatocytes, spotty necrosis, hydropic degeneration, liver oedema, congestion, swelling, vacuolisation, nuclear membrane collapse and overall cell death (Ali et al., 2019; Azim et al., 2015; Hassanein & El-Amir, 2017; Shirdare et al., 2022; Wang et al., 2013). TiO₂ NPs cause significant disruption to the liver structure with neutrophilic cell, portal and lobular infiltration by inflammatory cells and congested dilated central veins (Ali et al., 2019). TiO₂ NPs also cause necrosis, oedema, Kupffer cell hypertrophy, hydropic degeneration, vacuolisation in hepatocytes and

increased infiltration of inflammatory cells (Moradi et al., 2019) (see Tables 1 and 2 for more details). In vitro studies on culture human and rat liver cells have also shown cytotoxic effects of TiO₂ NPs (Sha et al., 2011). Similarly, TiO₂ NPs induced alterations in cell viability, increased ROS, decreased GSH levels in human hepatocarcinoma cell line (HepG2 cells) and also resulted in distorted cellular morphology in the liver (Abbasi-Oshaghi et al., 2019). In addition, cell growth inhibition, increased apoptosis, cell cycle arrest at G1 stage and induction of ROS-mediated ER stress by activating PERK/ATF6/Bax axis were observed in HepG2 cells (Li et al., 2020).

9 | OXIDATIVE, MITOCHONDRIAL AND ER STRESS IN THE LIVER

TiO₂ NP toxicity induces oxidative stress, increases in ROS formation and promotes inflammation (Foroozandeh & Aziz, 2015; Liguori

TABLE 2 Reported effects of TiO₂ NPs on liver (in vivo).

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	Effects on liver	References
CD-1 mice (M, F)	5000/single dose	1 day	NG/25, 80, and 155 nm	↑ALT/AST ratios HC: Ti accumulation in the liver, hydropic degeneration, spotty necrosis of hepatocytes	Wang et al. (2007)
CD-1 mice (F)	62.5, 125, 250	30 days	Anatase/5 nm	↑ ALT, ALP, AST, LDH, ChE, TP, TG, TCHO, NO ↓ ALB to GLB (A/G), TBIL, IL-2 HC: Blur hepatocytes, congested interstitial vessels	Duan et al. (2010)
CD-1 mice (F)	5, 10, 50	60 days	Anatase/6–7 nm	↑ H ₂ O ₂ , MDA, NO, O ₂ ⁻ , CYP1A ↓ SOD, CAT, GSH-Px, MT, GST, HSP70, p53, TF genes HC: Mitochondrial swelling, apoptotic bodies, chromatin condensation	Cui et al. (2010)
CD-1 mice (F)	5, 10, 50	60 days	Anatase/5 nm	↑ ALT, AST, ALP, LDH, PChE, LAP, TLR2, TLR4, IKK1, IKK2, NF-κB, NF-κBP52, NF-κBP65, TNF-α, NIK ↓ IκB, IL-2, IgM HC: TiO ₂ accumulation in the liver, fatty degenerations with large vacuoles and congestion, necrosis, inflammatory cell infiltration, mitochondria swelling, apoptotic cell with chromatin condensation	Cui et al. (2011)
Sprague-Dawley rats (M)	10, 50, 200	30 days	Anatase/75 nm	↑ Glu, LDL-C, ALT/AST ratio, BUN, GSH/GSSG ↓ AST, HBDH, CK, TBIL HC: Liver injury, oedema, hepatic cord disarray, peri-lobular cell swelling, hydropic degeneration, inflammatory cell infiltration	Wang et al. (2013)
Albino rats (M)	1200	9 months	Anatase/25–70 nm	↑ GPT, GOT ↓ MDA, GSH HC: Necrosis, hydropic degeneration and dead hepatocytes	Attia et al. (2013)
Swiss albino mice (M)	10, 50, 100	14 days	Anatase/20–50 nm	↑ ALT/AST ratio, ALT, AST, ALP, MDA, ROS, Hsp60, Hsp70, p53, Bax, caspases 3 and 9 ↓ GSH, Bcl-2 HC: Increased liver weight, accumulation of mononuclear cells near the sinusoidal vesicle, angiectasis	Shukla et al. (2014)
Wistar rats (M)	300	14 days	NG/50–100 nm	↑ AST, ALT, ALP, MDA ↓ GPx, SOD HC: Centrilobular necrosis, congestion, swelling, and vacuolisation, inflammatory cell infiltration, high apoptotic index	Orazizadeh et al. (2014)
CD-1 mice (M)	2.5, 5, 10	6 months	Anatase/5–6 nm	↑ ALT, AST, ALP, LDH, TC, TG, IL-4, IL-5, IL-12, IFN-γ, GATA3, GATA4, T-bet, STAT3, STAT6, eotaxin, MCP-1, MIP-2 genes ↓ STAT1, TBIL HC: TiO ₂ accumulation in the liver, angiectasis, hyperaemia, infiltration of	Hong et al. (2014)

(Continues)

TABLE 2 (Continued)

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	Effects on liver	References
				inflammatory cells, macrophage aggregation, hepatic tissue crevice, necrosis, apoptosis, mitochondrial swelling, nuclear membrane collapse and chromatin marginalisation	
Swiss albino mice (M)	500	21 days	Anatase/rutile/50–75 nm	↑ ROS, GSH, TBARS ↓ SOD, CAT, GPX HC: TiO ₂ particles entrapping in endosomes and Kupffer cells	Shrivastava et al. (2014)
Sprague-Dawley rats (M)	10, 50, 200	30 days	Anatase/75 nm	↑ T-SOD, GSH/GSSG ratio, Rb, ALT, AST, ALP, MDA ↓ Mo, co, Mn, P, GPx, SOD	Wang (2014)
Wistar rats (M)	300	14 days	NG/<100 nm	HC: Damaged lobular structures, vacuolisation and congestion, inflammatory cell infiltration	Khorsandi et al. (2015)
CD-1 mice (M)	64	28 days	Anatase/18 and 120 nm	↑ MDA, TNF- α , IL-6 ↓ T-SOD, GSH No effects on plasma glucose and ROS levels HC: Fracture in tissue fibres	Gu et al. (2015)
Albino mice (M)	150	14 days	Anatase/21 nm	↑ ALT, AST, MDA, TNF- α , IL-6, Nrf2, NF- κ B, CD68, Bax, caspase-3 ↓ GSH, Bcl-2 HC: Focal degeneration, necrosis, DNA damage, inflammatory cell infiltration	Azim et al. (2015)
CD-1 mice (M)	13, 64, 320	14 weeks	Anatase/25 nm	↑ MDA, JNK1, p38 MAPK, TNF- α , IL-6, IR, plasma glucose ↓ SOD, GSH ↑ TiO ₂ accumulation in the liver with increasing dose	Hu et al. (2015)
Albino rats (M)	5000	Every other day for 60 days	NG	↑ AST, ALT, LDH, TLR-2, TLR-4, NF- κ B/p65, CYP1B1 and CYP2B HC: Aggregation of macrophages, hepatocytes with large nuclei and intracytoplasmic vacuoles mild congestion of the portal blood vessels, hydrophobic degeneration, and lymphocytic aggregations in portal areas	Moustafa and Hussein (2016)
Sprague-Dawley rats (M)	150	6 weeks	NG/21 nm	↑ AST, ALT, LPO, TNF- α ↓ GSH, TBARS HC: Congestion and dilatation, degenerative changes, vacuolar degeneration, necrosis, mononuclear infiltration	Hassanein and El-Amir (2017)
C57/BL6 mice (M)	250, 500	14 days	NG/21 nm	↑ IL1B, TBIL, ALP, TBA, Oapt1, Mrp3, Cyp2b10, 2c37 HC: Number of mitochondria increased and oedema in ER	Yang et al. (2017)
Kun Ming mice (M, F)	2000	7 days	Anatase/25 nm	↑ AST, ALT, TBIL, MDA ↓ SOD, GSH-PX, Nrf2, NQO1, HO-1, GCLC HC: Dilatation of sinusoids, deranged and swollen hepatocytes, vacuoles, necrosis	Niu et al. (2017)

TABLE 2 (Continued)

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	Effects on liver	References
ICR mice (M, F)	5, 10, 50	60 days	Anatase/10, 60, 90 nm	<p>↑ ALP, ALT, ALB, LAP, PChe, TBIL, TP, O₂⁻, H₂O₂, NO, MDA, CYP1A</p> <p>↓ SOD, CAT, MT, GST, HSP70, p53, TF, GSHPx</p> <p>HC: TiO₂ accumulation in the liver, vascular obstruction, dilation, increase basophils, partial ischemia, swelling mitochondria, vacuoles in mitochondria, nucleolus collapse, scattered chromatin, apoptosis</p>	Jia et al. (2017)
Sprague-Dawley rats (M)	150	28 days	Anatase/30–80 nm	<p>↑ ALP, ALT, AST, LPO</p> <p>↓ CAT, GST</p> <p>HC: Destructed blood vessels, infiltration of neutrophils and apoptosis, damaged and congested vein, vacuolation, haemorrhage, pyknotic nuclei, dilated sinusoids</p>	Shakeel et al. (2018)
Sprague-Dawley rats (M)	300	14 days	NG	<p>↑ AST, ALT, LPO</p> <p>↓ TBARS</p> <p>HC: Congestion of the central veins, dilatation of the hepatic sinusoids, focal haemorrhagic, coagulative necrosis, proliferation of Kupffer cells, lymphocytic aggregations, vacuolar degeneration</p>	Hassanein and El-Amir (2018)
Albino rats (M)	100	60 days	Anatase/10 nm	<p>↑ ALT, AST, ALP, MDA, Bax</p> <p>↓ GPx, SOD, GSH, Bcl-2</p> <p>HC: Hepatic apoptosis, hepatocellular necrosis, steatosis, sinusoidal dilation with leucocytosis, distortion, disorganisation of the hepatic cords</p>	Morgan et al. (2018)
Wistar rats (M)	100, 200	60 days	Anatase/40 nm	<p>↑ ALT, AST, ALP, ALB, MDA</p> <p>↓ SOD, GPx, CAT, GSH</p> <p>HC: Liver tissue damage, sinusoidal dilation, vacuolisation and leucocyte infiltration</p>	Jafari et al. (2018)
Wistar albino rats (M)	1000	21 days	Anatase 60 nm	<p>↑ ALT, TNF-α, IL-6, CRP, IgG, NO, VEGF, caspase-3, LPO</p> <p>↓ cytochrome-P450</p> <p>HC: Severe degeneration, nuclear pyknosis, karyolysis, cytoplasmic vacuolation, increase in collagen, DNA damage</p>	Fadda et al. (2018)
Wistar albino rats (F)	0.5, 5, 50	14 days	NG/21 nm	<p>↑ CAT</p> <p>↓ GST, SOD, GPX</p> <p>HC: TiO₂ accumulation in the liver</p>	Canli et al. (2019)
Sprague-Dawley rats (M)	2, 10, 50	90 days	Anatase/29 nm	<p>↑ TP, ALB, GLB, GSSG, MDA, IL-1α, IL-4, TNF-α</p> <p>↓ GSH, GSH-Px, SOD</p> <p>HC: Fatty degeneration, fat vacuoles, vacuolation of mitochondria, changes hepatic metabolomics, disrupted energy metabolism</p>	Chen et al. (2019)
Wistar rats (M)	10, 50, 100	30 days	Rutile/30 nm	<p>↑ ALT, AST, LDH, ALP, MDA, TOS, Bax, p53, NLRP3, caspase 1 and 3, IL-1β, TNF-α, iNOS</p> <p>↓ SOD, GPX, CAT, TAC, Bcl-2</p>	Abbasi-Oshaghi et al. (2019)

(Continues)

TABLE 2 (Continued)

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	Effects on liver	References
				HC: Liver cell degeneration, necrosis, congestion of the sinusoids, lymphocytic infiltration, deposition of collagen, apoptosis	
Wistar rats (M)	300	14 days	80% anatase + 20% rutile/20 nm	<p>↑ ALT, AST, ALP, LDH, MDA, TOS, TNF-α, NF-κB</p> <p>↓ SOD, GPx, CAT, TAC</p> <p>HC: Dilatation of congested portal vein, hypertrophy of Kupffer cells, hydropic and vacuolar degeneration, oedema and necrosis and inflammatory cell infiltration</p>	Moradi et al. (2019)
Swiss albino mice (M)	50, 250, 500	5 days	NG/21 and 80 nm	<p>↑ AST, ALT, MDA, NO, CAT</p> <p>↓ GSH</p> <p>HC: Chromosomal fragments and dilatations, distorted and loss in lobular architecture, micro regenerating nodules, mild ballooning and infiltration by lymphocytes, mild fibrosis, swelling and degeneration, significant haemorrhage</p>	Ali et al. (2019)
NFR mice (M)	5	21 days	Anatase E171/201 nm	<p>↑ accumulation of TiO₂ in the liver, TNF-α, IL-1β, circulatory cytokines (IL-6, SDF-1)</p> <p>↓ IL-10</p> <p>HC: Increased necroinflammatory foci infiltrated with F4/80-positive cells, macrophage recruitment</p>	Talamini et al. (2019)
CD-1 mice (M)	50	8 and 26 weeks	NG/25 nm	<p>↑ Cyp2b9, Cyp2c70, Cyp4a14, GRP78, CHOP, PERK, p-eIF2α, GRP78, CHOP, XBP1-s, ATF6, XBP1-s/XBP1-t, Nrf2, Nqo1, (HO-1), NF-κB</p> <p>Activation of MAPK pathways, IR and increase plasma glucose level</p>	Hu et al. (2020)
Mice	2.5, 5, 10	9 months	NG	<p>↑ HIF-1α, Wnt3, Wnt4, NF-κB, TGF-β1, TGF-β1R, Smad-2, ILK, ECM, calpain 2, α-SMA, c-Myc, collagen I, p38 MAPK phosphorylation, GSK-3β, β-catenin</p> <p>↓ cyclin D</p> <p>HC: Hepatic inflammatory cell infiltration, hepatic fibrosis</p>	Hong et al. (2020)
Sprague-Dawley rats (M)	2, 10, 50	28 days	Anatase/25 nm	<p>↑ MDA, TNF-α, SOD</p> <p>HC: Liver is the most sensitive organ to nano-TiO₂-induced oxidative/antioxidant biomarker changes</p>	Zhou et al. (2020)
Wistar rats (M)	300	14 days	NG/50–100 nm	<p>↑ ALT, AST, Bax</p> <p>↓ Bcl-2</p> <p>HC: Apoptosis in all lobules</p>	Orazizadeh et al. (2020)
Sprague-Dawley rats (M)	2, 10, 50	90 days	Anatase/29 nm	<p>↑ GSSG, MDA</p> <p>↓ GSH, GSH/GSSG, SOD, hepatic phosphatidylcholine (PC)</p> <p>HC: High oxidative stress, fatty degeneration of hepatocytes, altered lipid metabolism</p>	Chen et al. (2020)
Albino rats (M)	500	14 days	NG/63–142 nm	<p>↑ ALT, ALP, MDA, TLR-4, NF-κB, NIK, TNF-α</p>	Mohammed and Safwat (2020)

TABLE 2 (Continued)

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	Effects on liver	References
				↓ GSH, CAT, acetylcholinesterase HC: Congested and dilated central vein, vacuolated and degenerated hepatocytes with Kupffer cells, leucocytes infiltration, endothelial hyperplasia, leucocytic aggregates	
Sprague-Dawley rats (F)	50	21 days	NG/100 nm	↑ ALT, AST, T.BIL, D.BIL, creatinine, urea, uric acid, Cho, TG, LDL-Cho, MDA, NO, Bax, TNF- α ↓ TP, Alb, HDL-Cho, CAT, SOD, GPx, Bcl-2 HC: Portal vein dilatation, congestion, periportal necrosis, proliferative bile ducts, aggregation of mononuclear cellular infiltration and fibrous tissues	Abdel-Wahhab et al. (2021)
Kunming mice (M)	2, 20	8 weeks	Anatase/10–25 nm	↑ ALP, TNF- α , IL-1 β , IL-6, TLR-4, caspases 3 and 9, VEGFA, TGF- β , IFN- γ , MDA ↓ SOD, CAT, GSH HC: Hepatocyte swelling, fat accumulation, inflammatory infiltration	Zhao et al. (2021)
Sprague-Dawley rats (F)	150	7 days	NG/33 nm	↑ AST, ALT, AST/ALT, ALP ↓ SOD1, SOD2, HO-1, GSH, CAT, GCLC, GCLM HC: Ti accumulation in the liver, disordered arrangement of hepatocytes, ballooning degeneration	Nie et al. (2021)
Wistar rats (M)	100	30 days	NG/21 nm	↑ LDH, ALT, AST, TC, TG, LDL, VLDL, plasma glucose ↓ HDL HC: Congestion and dilatation in the central vein	Bakour et al. (2021)
Balb/c mice (M)	25	21 days	NG/28.9 nm	↑ ALT, AST, TC, TG, LDL, MDA, NO, AFP, TNF- α , CEA ↓ CAT, SOD, TP, Alb, TAC, HDL HC: DNA fragmentation, hepatocytes necrosis, dilated and congested blood vessels, fatty droplets	Salman et al. (2021)
Sprague-Dawley rats (M)	50	21 days	NG/28 nm	↑ AST, ALT, MDA, NO, TG, Chol, LDL, Bax, caspase 3, p53 ↓ HDL, GPx, CAT, Bcl-2 HC: Portal tract dilation, proliferation of bile ducts, necrosis in their epithelial cells, fibrosis, DNA fragmentation in hepatic tissue	Sallam, Ahmed, El-Nekeety, et al. (2022)
Wistar rats (M)	300	21 days	Anatase (80%), rutile (20%)/20 nm	↑ ALT, AST, ALP, LDH, TOS, MDA ↓ TAC, SOD, GPx HC: WBCs infiltration, central vein hyperaemia, enlargement of Kupffer cells, dilation of sinusoids, inflammatory cells accumulation, hepatocyte necrosis	Shirdare et al. (2022)
Sprague-Dawley rats (M)	50	21 days	NG/50 nm	↑ AST, ALT, T.BIL, D.BIL, Cho, TG, LDL-Cho, AFP, CEA, NO, MDA, IL-1 β , IL-6, TNF- α ↓ Alb, TP, HDL-Cho, CAT, GPx, SOD, IL-10	Sallam, Ahmed, Diab, et al. (2022)

(Continues)

TABLE 2 (Continued)

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	Effects on liver	References
				HC: Dilatation in the central vein and hepatic sinusoid, vacuolar degeneration, nuclear degeneration, necrosis, pyknosis, karyolysis, peripheral chromatin clumps, fibrosis, proliferation of bile ducts	
Sprague-Dawley rats (M)	2, 10, 50	90 days	Anatase/29 nm	<p>↑ MDA, imbalance in the liver metabolites, effects on glycerophospholipid metabolism pathway, effect on the liver metabolism</p> <p>↓ differentially expressed phosphatidylcholine (PCs)</p>	Chen et al. (2022)
Wistar rats (M)	100	28 days	NG	<p>↑ AST, ALT, ALP, TC, TG, MDA, IL-1β, IL-6, TNF-α, IFN-γ, γH2A, Bax, α-SMA, fibronectin</p> <p>↓ CAT, GPx, SOD, GSH, IL-10, Bcl-2, PI3K/AKT signalling pathway</p> <p>HC: Hepatic fibrosis, inflammatory cell infiltration, hepatic sinusoid congestion, widening liver tissue gap and lamellar tissue necrosis</p>	Zhang et al. (2023)
ICR mice (M)	50	30 days	Anatase/7 nm	<p>↑ AST, ALT, ALP, MDA, Bax, caspase-3 & 9, p53, p-p38, p-p38/p38</p> <p>↓ SOD, GSH-Px, GSH, T-AOC, Bcl-2</p> <p>HC: Hepatocytes were blurred and disordered, swollen and vacuolated, increased apoptosis, steatosis, congestion and dilation of the central veins and infiltration of inflammatory cells around the blood vessels</p>	Chang et al. (2023)
Wistar rats (M)	20	14 days	Anatase/25 and 150 nm	<p>↑ IL-1β</p> <p>↓ IL-6 and IFN-γ</p> <p>HC: Increase in liver weight, dilated hepatic vein, loss of native morphology of hepatic lobule and portal triad, structural deformities in RBCs found in liver</p>	Ali et al. (2023)
Sprague-Dawley (M, F)	1, 2	5 days	Anatase/<25 nm	<p>↑ genes like NPY and SPP1 (male rats only)</p> <p>AST and ALT (no effect)</p> <p>HC: Increase in intralobular lymphoid infiltration, focal intralobular necrosis in the middle zone of the liver lobule, hepatocyte vacuolisation/steatosis, congestion in the central vein with enlargement of the sinusoids</p>	Tassinari et al. (2023)
Wistar rats (M)	100	28	NG	<p>↑ AST, ALT, ALP, TC, TG, MDA, IL-6, TNF-α, IFN-γ, IL-1β, TNF-α, γH2A, Bax, α-SMA, fibronectin</p> <p>↓ SOD, GPx, GSH, CAT, IL-10, Bcl-2</p> <p>HC: Hepatic fibrosis, hepatic sinusoid congestion, widening liver tissue gap, inflammatory cell infiltration, lamellar tissue necrosis, and impairment of the PI3K/AKT signalling pathway</p>	Zhang et al. (2023)

TABLE 2 (Continued)

Study model	Oral daily dosage (mg/kg/bw)	Treatment timeline	Type/particle size	Effects on liver	References
Sprague-Dawley rats (M)	250	28	Anatase/15 nm	↑ TOS, MDA ↓ TAS, CAT HC: Mononuclear cell infiltration, hyperaemia, glycogen accumulation, fibrosis proliferation, hepatocellular hypertrophy	Ogut et al. (2024)
Albino rats (M)	500	60	Anatase/72.1 nm	↑ CYP1A1 and NBN	Moselhy et al. (2024)
Swiss Webster mice (M)	50	5	Anatase and rutile mixture/NG	↑ damage to genomic DNA, p53, MDA ↓ SOD, Gpx HC: Diffusion and degeneration of hepatocytes.	Mohamed et al. (2024)
C57BL/6 mice (F)	10	28	NG/25–70 nm	↑ body weight, liver weigh, Ti content in liver, AST, ALT, AKP, MDA, <i>keap-1</i> ↓ GSH, CAT, SOD, <i>Nrf2</i> , <i>Gclc</i> , <i>Gclm</i>	Jia et al. (2024)

et al., 2018). ROS generation results in the disruption of macromolecules (lipids, DNA, carbohydrates and proteins) (Abdel-Wahhab et al., 2021; Shukla et al., 2014). Lipid peroxidation causes structural changes in the cell membrane and disturbs the overall functions of the cell. Due to lipid disturbances, free radicals (hydroxyl radicals) are generated that cause oxidative damage by increasing the levels of MDA and NO and decreasing hepatic antioxidant enzymes (GSH, CAT, SOD, GPx) (Chen et al., 2020). TiO₂ NPs increase levels of oxidative stress and MDA and a decrease in the expression of antioxidant genes (SOD, CAT, GSH) (Jafari et al., 2018). Oral ingestion of TiO₂ NPs in rats induces oxidative stress and causes a slight elemental imbalance in the liver (Chen et al., 2020).

Mitochondria are one of the main organelles targeted by TiO₂ NPs where they cause alterations in mitochondrial membrane proteins, mitochondrial dynamics and morphology (Hirakawa et al., 2004). TiO₂ NPs cause morphological alterations by damaging mitochondrial membranes, swelling and ballooning of mitochondria and overall decrease the activity of mitochondria in hepatic cells (Chen et al., 2019; Jia et al., 2017; Teubl et al., 2015; Yang et al., 2017). TiO₂ NPs alter the activity of electron transport chain components, including mitochondrial complex I (nicotinamide adenine dinucleotide [NADH] dehydrogenase) and complex II (succinate dehydrogenase). Alterations in these enzymes result in defective oxidative metabolism that can lead to mitochondrial cytopathy. Also, changes in these complexes may result in an aberrant mitochondrial permeability transition pore, further causing uncoupling of oxidative phosphorylation and depletion of ATP (Mehndiratta et al., 2002). Waseem et al. (2022) have shown that TiO₂ NPs drop the activity of mitochondrial complex I and complex II mitochondrial dehydrogenase (complex III) in the rat liver. Also, TiO₂ NPs aggravated membrane peroxidation, decreased the activity of Mn-SOD (which is the first line of defence against the toxic oxyradicals), reduced the levels of GSH (crucial for maintaining mitochondrial functionality) and reduced

mitochondrial GST (responsible for inactivating the cytotoxic effects of oxidative stress).

The endoplasmic reticulum (ER) controls the accurate folding of proteins. Accumulation of misfolded proteins in the ER causes ER stress, which activates an unfolded protein response (UPR). The UPR either maintains homeostasis or triggers cell death to inhibit accumulation of damaged cells (Cao et al., 2017). The UPR has three signal transducers: protein kinase RNA-like ER kinase (PERK); inositol-requiring enzyme 1 alpha/beta (IRE1 α/β); and activating transcription factor 6 (ATF6). During ER stress, PERK (ER-resident protein) mediates signal transduction and, together with ATF6 and IRE1, triggers either ROS-ER stress-mediated apoptosis or autophagy (Li et al., 2022; Liu et al., 2015). Nanoparticle-induced ER stress is one of the early biomarkers for the evaluation of nanotoxicity. TiO₂ NPs increase the expression of PERK, Bax and ATF6 in HepG2 cell lines. TiO₂ NPs induce ER stress that activates PERK/ATF6 signalling pathways contributing to the TiO₂-mediated apoptosis of the liver cancer cells (Li et al., 2020). The PERK-eIF2 α -ATF4 pathway up-regulates the UPR target genes and induces the folding and excretion of proapoptotic protein C/EBP homologous protein (CHOP), which regulates both lipogenesis and hepatic steatosis (Hernández-Gea et al., 2013). Thus, TiO₂ NPs can induce ER stress in the liver cells; however, further studies are needed to elucidate the role of TiO₂ NPs in mediating ER stress, apoptosis and autophagy in the liver cells.

10 | INFLAMMATION, APOPTOSIS AND NECROSIS IN THE LIVER

Oral administration of TiO₂ NPs causes chromatin condensation, nuclear fragmentation and apoptosis in hepatocytes (Cui et al., 2010; Zhu et al., 2012). TiO₂ NPs increase the expression of pro-apoptotic

genes like p53, Bax, Cyto-c, Apaf-1, caspase-9 and caspase-3, and reduce the expression of anti-apoptotic genes like Bcl-2, indicating that TiO₂ NPs mediate apoptosis via the caspase-dependent signalling pathway. This pathway has been demonstrated in vitro in HepG2 cells (Shukla et al., 2013), while in vivo it was confirmed in the liver of rats using the TUNEL assay (Abbasi-Oshaghi et al., 2019). Similar studies using immunoblot analysis also revealed that TiO₂ NPs activate the intrinsic pathway of apoptosis by increasing the expression of pro-apoptotic proteins and decreasing the levels of anti-apoptotic protein (Sallam, Ahmed, Diab, et al., 2022; Sallam, Ahmed, El-Nekeety, et al., 2022; Shukla et al., 2014). TEM studies have shown that TiO₂ NPs cause the breakdown of nucleolus, dispersed chromatin, apoptosis and apoptotic cell bodies in the liver cells of mice (Jia et al., 2017). This shows that TiO₂ NPs induce liver apoptosis as well as structural damage in the liver.

Another study reported that TiO₂ NPs increase pro-apoptotic factors (Bax, caspase-3 and p53) and decrease anti-apoptotic factor (Bcl-2) in the rat liver (Abbasi-Oshaghi et al., 2019). The caspases and Bcl-2 proteins are the main regulators of the apoptotic pathway. Translocation of Bax into mitochondria alters the permeability of cytochrome C and further stimulates post-mitochondrial caspases that lead to apoptotic cell death (Jin et al., 2017; Wang, 2014). Necrosis is one of the primary features of liver injury and up-regulates the expression of apoptotic genes (p53, p38, TNF- α , caspase-3, caspase-8, caspase-9). This suggests the role of TiO₂ NPs in causing cell necrosis through ROS (Abbasi-Oshaghi et al., 2019; Moradi et al., 2019; Morgan et al., 2018; Sallam, Ahmed, Diab, et al., 2022).

TiO₂ NPs activate MAPK and nuclear factor kappa B (NF- κ B) inflammatory signalling cascades, leading to transient liver inflammation due to increased levels of pro-inflammatory cytokine tumour

necrosis factor α (TNF- α) and decreased expression of NF- κ B inhibitor A20 (anti-inflammatory gene) and decreased cell viability (Chen et al., 2016). In addition, oral administration of TiO₂ NPs causes infiltration of white blood cells, inflammation with infiltration of white blood cells, Kupffer cell enlargement, sinusoidal dilation with the accumulation of inflammatory cells and hepatocyte necrosis (Shirdare et al., 2022). Abbasi-Oshaghi et al. (2019) have reported that TiO₂ NPs induce inflammatory responses by increasing the expression of TNF- α (a pro-inflammatory cytokine) and iNOS (major source of reactive nitrogen species) levels dose-dependently in the liver. Zhao et al. (2021) have shown that high dose oral intake of TiO₂ NPs causes severe oxidative stress, serious hepatic inflammation, fibrosis and apoptosis in the liver of mice, and these effects were exacerbated with fructose-induced metabolic syndrome. These results suggest that some populations, such as those with metabolic syndrome, may be more at risk of adverse health outcomes associated with TiO₂ NPs.

Overall, these studies suggest that TiO₂ NPs induce necrosis, apoptosis, inflammation and other liver impairments via ROS generation, DNA damage, ER stress and mitochondrial stress (Mohammadinejad et al., 2019). The molecular mechanisms by which TiO₂ NPs induce apoptosis, necrosis and inflammation have been summarised in Figures 3 and 4. However, the role of TiO₂ NPs in molecular signalling pathways towards autophagy in the liver cells remains elusive.

11 | GENOTOXICITY IN THE LIVER

Genotoxicity is defined as the ability of biological or chemical agents (harmful substances) to damage the genetic information in the cells

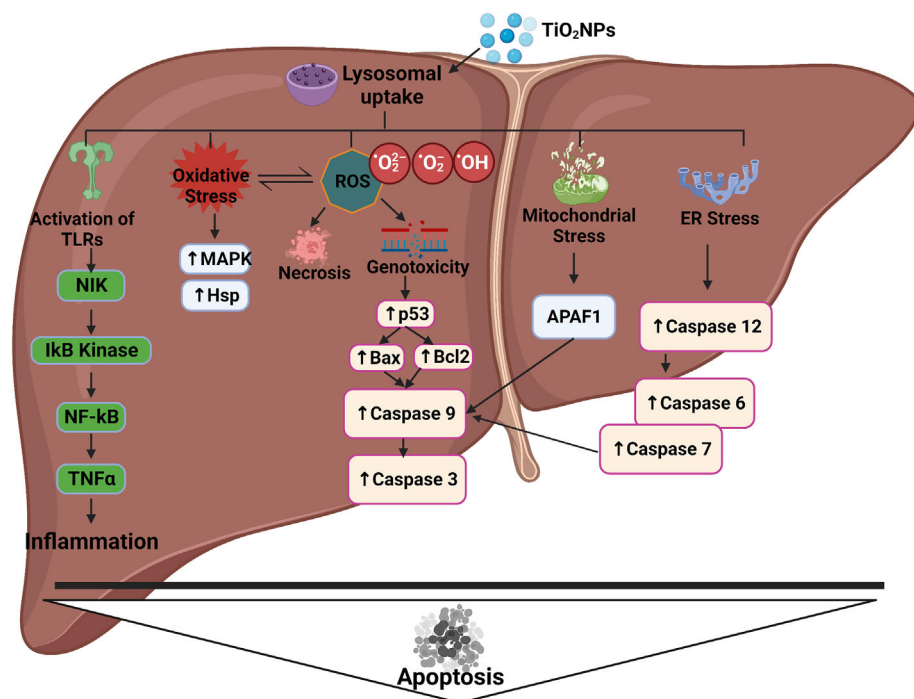


FIGURE 3 Mechanisms of hepatotoxicity induced by TiO₂ nanoparticles (NPs). TiO₂ NPs activate Toll-like receptors (TLRs) and enhance NF- κ B-inducible kinase (NIK), leading to nuclear factor-kappa B (NF- κ B) response to cause cellular inflammation. TiO₂ NPs induce oxidative stress, generate ROS species and increase the expression of stress protein Hsp (heat shock protein) and activate MAPK (mitogen-activated protein kinases) signalling pathway. TiO₂ NPs increase the expression of p53 that is involved in increasing the expression of Bax and Bcl2 subsequently increasing the expression of caspases 3 and 9, resulting in apoptosis. TiO₂ NPs also induce mitochondrial and ER stress mediated by apoptotic protease activating factor 1 (APAF1) and caspases (caspases 12, 6 and 7). Created with BioRender.

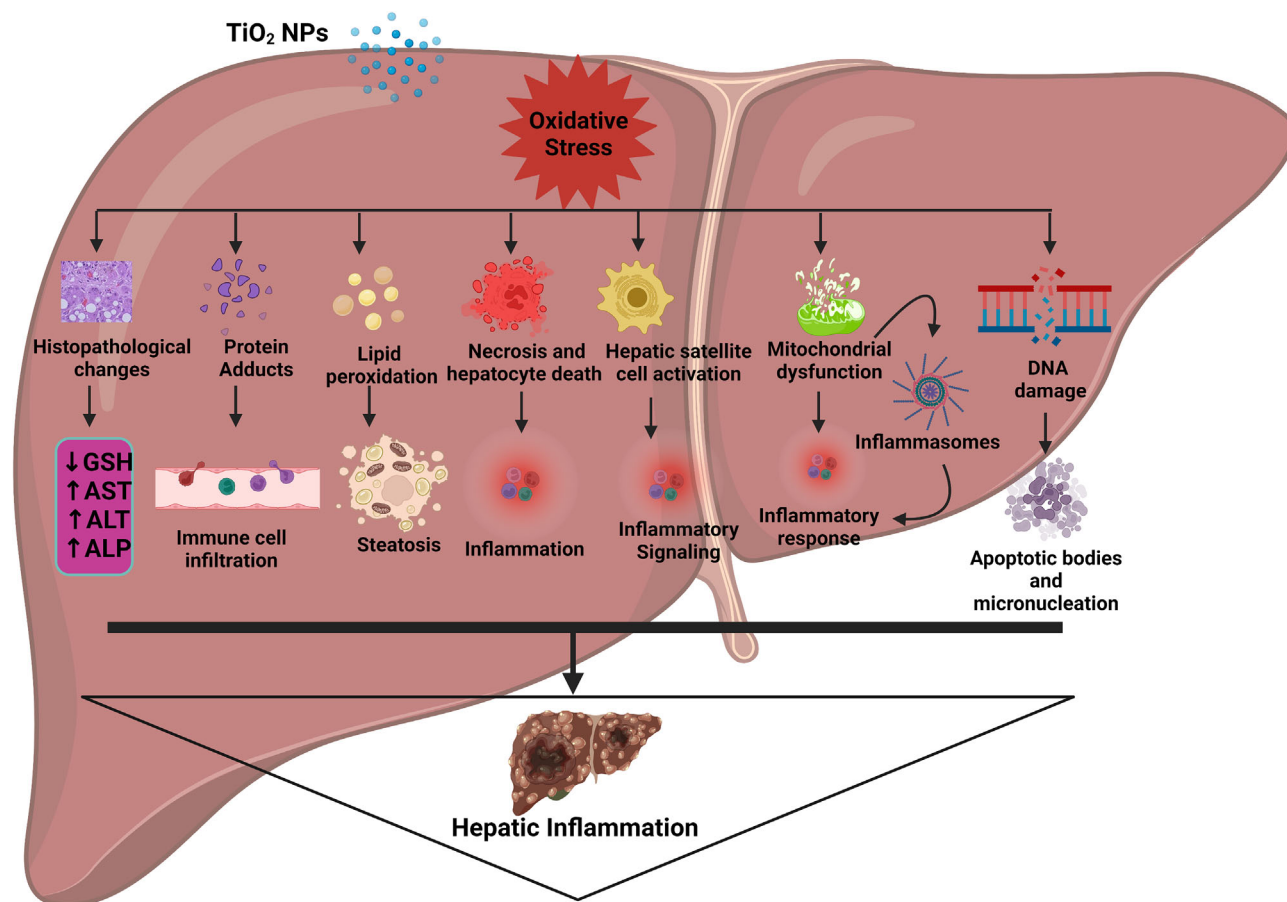


FIGURE 4 TiO₂ NP-induced oxidative stress triggers inflammation. TiO₂ NPs induce oxidative stress and cause hepatic inflammation by changing the histopathology of the liver, generation of protein adducts, lipid peroxidation, necrosis, hepatocyte death, mitochondrial dysfunction and DNA damage. Created with BioRender.

(directly or indirectly) triggering genomic instability and mutations that may cause various diseases including cancer (Phillips & Artl, 2009). Oral exposure to TiO₂ NPs causes DNA damage, increases the expression of pro-apoptotic proteins (Bax) and p53 and decreases the expression of anti-apoptotic proteins (Bcl-2), further leading to apoptosis and distorted liver function (Abdel-Wahhab et al., 2021; Orazizadeh et al., 2020; Sallam, Ahmed, El-Nekeety, et al., 2022; Shukla et al., 2014). In addition, TiO₂ NPs also induced chromosome breaks and polyploidy. In vivo studies have confirmed that TiO₂ NPs cause chromosomal aberrations and DNA breaks and can induce genotoxicity in the liver, spleen and thymus cells (Ali et al., 2019; Chakrabarti et al., 2019; Manivannan et al., 2020). For more details on TiO₂ NP-induced genotoxicity, see Wani and Shadab (2020).

12 | CHALLENGES AND PERSPECTIVES

E171 is a widely used additive in food and pharmaceutical formulations. Recently, the usage of E171 in food and pharmaceutical products was banned in the EU due to its potentially genotoxic effects (Younes et al., 2021). Subsequently, the use of E171 in food was banned in several other countries, including Jordan, Saudi Arabia,

Yemen, Qatar, Turkey and Israel. However, other countries including the United States, the United Kingdom, Canada, Japan, Australia and New Zealand are still using E171, as the Food and Drug Administration (FDA), the UK Food Standards Agency (FSA), Health Canada, Japanese National Institute of Health Sciences (NIHS) and Food Standards Australia New Zealand (FSANZ) determined that there is no conclusive scientific evidence that E171 is harmful to human health (FSANZ, 2022; TDMA, 2023). The different regulatory responses to the same evidence are presumably due to different risk tolerance thresholds. For example, France took precautionary regulatory action and banned the usage of E171 in 2019, because the risk to public health could not be conclusively ruled out. Later, following a similar approach, the use of E171 as a food additive was banned in the EU. The EFSA concluded that risk associated with E171, primarily genotoxicity, could not be ruled out. In contrast, other countries have not implemented any restrictions or bans because their regulatory agencies require more conclusive evidence of risk to human health before adding restrictions to products already in use.

Orally consumed TiO₂ NPs are mostly excreted but some get absorbed via the small intestine and accumulate in various organs including liver, which is the primary detoxification system of the body (Gilbert et al., 2021; Hamilton, 2013; Heringa et al., 2018; Keller

TABLE 3 Effects of TiO₂ NPs on liver cells (in vitro).

Cell type	Dosage (µg/ml)	Treatment timeline (h)	Type/size	Effects on liver cells	References
HepG2	1, 10, 100, 250	4, 24, 48	Anatase/<25 nm Rutile/<100 nm	Anatase: 2-fold increase in ROS Persistent DNA stranded breaks ↑ Fpg-sensitive sites, p53, mdm2, p21, gadd45α Rutile: 1.4-fold increase in ROS, DNA-stranded breaks but not persistent ↑ Fpg-sensitive sites, p53, mdm2, p21, gadd45α	Petković et al. (2011)
SMMC-7721 HL-7702	0.1, 0.5, 1, 5, 10, 50, 100	12, 36, 24, 48	NG/3.7 nm	↑ cell shrinkage, nuclear condensation, ROS, cytotoxicity ↓ GSH	Sha et al. (2011)
C3A	0.5–256	4, 6, 24	Rutile/7 and 10 nm	↑ DNA damage, ROS, oxidative stress, genotoxicity, IL8 ↓ GSH	(Kermanizadeh et al., 2012)
HepG2	1, 10, 20, 40, 80	6, 24, 48	Anatase/30–70 nm	↑ cellular uptake, cytotoxicity, oxidative DNA damage, apoptosis, micro nucleated cells, ROS, Hsp60, Hsp70, p53, Bax, Cyto-c, Apaf-1, caspases 3 and 9, hydroperoxide ↓ MSD, GSH, MMP, Bcl-2	Shukla et al. (2013)
HepG2	100	24	Anatase 86% Rutile 14%/sP25 (21 nm)	↑ DNA damage, MN frequencies, NF-κB activity No significant transcriptional activation of AP1	Prasad et al. (2014)
C3A	10–100	4	Rutile/30.5 nm	↑ ROS Limited effect on glycogen breakdown, glucose, LP release and metabolism	Filippi et al. (2015)
HepG2	5, 20, 80, 160, 320	1, 9, 12, 16, 24	Anatase/10 nm Rutile/50 nm	↑ cytotoxicity, immunogenicity, TNF-α, MAPK, NF-κB pathway, p38, ERK1/2 phosphorylation, inflammation, Young's modulus, and adhesion force. ↓ A20	Chen et al. (2016)
QGY	40, 80	72	NG/21 nm	↑ H ₂ O ₂ , chromosome instability, ROS ↑ telomere length, TRF1, TRF2, POT1, Nrf-2	Wang et al. (2018)
HepG2	2.5, 5, 7.5, 10	12, 24, 48	Anatase/10 nm	↑ G1 phase, caspases 3 and 7, apoptosis, VDAC1, Cyt c, αENaC, SIRT3, ADP/ATP, depolarisation and disruption of mitochondria ↓ cell growth and proliferation, S phase, ACS1, ATP	Xia et al. (2018)
HepG2	100, 150, 200, 300	24	Rutile/30 nm	↑ LDH, AST, ALT, ROS, apoptosis ↓ cell viability, GSH	Abbasi-Oshaghi et al. (2019)
HepG2	100	24, 72	P25/21 nm	↑ promoter methylation in CDKN1A, DNAJC15, GADD45A, GDF15, INSIG1, SCARA3, TP53, BNIP3 ↓ global methylation, DNMT3a, DNMT3b, MBD2, UHRF-1	Pogribna et al. (2020)
HepG2	10, 50, 100, 200	3, 24	Anatase (80%), rutile (20%)/25 nm	↑ NP uptake No micronuclei expression	Brandão et al. (2020)
HepG2	2.5, 5, 7.5, 10	48	NG	↑ apoptosis, cell cycle arrest at G1 stage, ROS, ER stress, PERK, ATF6, Bax ↓ cell growth	Li et al. (2020)
HepG2	4, 8, 12, 50	24	NG/21 nm NG/125 nm	↑ cytotoxicity, oxidative stress, TiO ₂ uptake ↓ fatty acid oxidation	Zhang et al. (2021)

TABLE 3 (Continued)

Cell type	Dosage ($\mu\text{g}/\text{ml}$)	Treatment timeline (h)	Type/size	Effects on liver cells	References
HepG2	1.56, 3.13, 6.25, 12.5, 25, 50, 100, 200	48	Anatase/25 nm	↑ cytotoxicity, epigenetic changes ↓ HADHB, BRIP1, ZNF562	Shi et al. (2023)

et al., 1995; Lima et al., 2004). Considering the potential effects of TiO₂ NPs on human liver health, all relevant literature (to date) reporting toxicity of TiO₂ NPs in the liver after oral administration in rodents and humans or in cultured liver cells has been summarised in this review. Briefly, most studies have shown that TiO₂ NPs can cause liver toxicity by various mechanisms including increasing oxidative stress, disturbing the antioxidant system, inflammation, apoptosis, necrosis and changing the expression levels of protective genes (Chen et al., 2020; Nie et al., 2021; Sallam, Ahmed, El-Nekeety, et al., 2022).

Similarly, orally administered TiO₂ NPs can induce toxicity in the liver of rodents from low doses, which are more relevant to daily human exposure, that is, 2–10 mg/kg/bw. Other studies have shown liver toxicity using doses well above normal human intakes, with doses as high as 50–2000 mg/kg/bw. In addition to dose ranges, a variety of particle sizes from 5 to 200 nm or even bulk particles can induce toxicity in the liver. These studies have also demonstrated that higher doses and smaller particle size are capable of causing more hepatotoxicity in rodents. Adverse effects have been reported following exposure to TiO₂ NPs in acute treatments for as low as 5 days and in chronic treatments for up to 9 months. The use of both crystalline forms of TiO₂, that is, anatase or rutile separately or a mixture of both, can elicit adverse effects on the liver (for details, see Tables 2 and 3).

In contrast, there are many studies that have reported very minor or no toxic effects of TiO₂ NPs in rodents (Akagi et al., 2023; Blevins et al., 2019; Han et al., 2021; Heo et al., 2020; Lin et al., 2023; Younes et al., 2021) (for details see Table 1). Research supporting the safety of TiO₂ raises some shortcomings in evidence suggesting adverse effects of TiO₂ NP administration. For example, the level or extent of toxicity of TiO₂ NPs is highly dependent on particle characterisation (size, form, purity, surface charge, particle distribution and stability in the experimental medium) and other factors including duration of exposure, its dose range, dosage relevancy to human, type of experimental model and sample size (Ali et al., 2019; Kassama & Liu, 2017; Violatto et al., 2023). Firstly, in terms of dose, most animal experiments have used doses higher than those encountered in typical human exposure scenarios to observe potential effects of TiO₂ NPs more quickly. Some studies have used doses of up to 2000–5000 mg/kg/bw, which are several orders of magnitude higher than the average daily intake in humans, and therefore, care must be taken in the extrapolation of these results to humans. Conversely, some studies have also shown adverse effects in the dose range of 1–5 mg/kg body weight, which is within the range of normal human exposure (0.2–3 mg/kg/bw) (Bischoff et al., 2021). This use of high

dose range may not intend to represent realistic human exposure levels but rather to provide a safety buffer in risk assessments.

There is a difficulty in modelling human TiO₂ consumption in animal studies. Studies in rats and mice most commonly deliver TiO₂ in a daily bolus dose via oral gavage. In contrast, human consumption of TiO₂ NPs occurs over time and is typically spread out across multiple meals and snacks, as individuals ingest products containing TiO₂ NPs as part of their daily diet. Delivery of substances via gavage is known to effect the absorption, bioavailability and metabolism compared to other oral routes (Mohammadparast & Mallard, 2023). Furthermore, the use of gavage can induce stress responses, which may alter the effect of a given chemical (Vandenberg et al., 2014). This difference in exposure patterns between animal models and humans should be considered when interpreting study results and extrapolating findings to human risk assessments. Although it is hard to achieve human eating pattern in rodents yet, instead of a single bolus, TiO₂ NPs can be administered in food to mimic human consumption pattern.

All other factors like study designs, type (nano or bulk particles) and crystalline form of TiO₂ NPs, exposure duration, methods of administration and endpoints assessed can impact the consistency and reproducibility of results. For instance, as discussed earlier, TiO₂ NPs can be in rutile or anatase or in a mixture form (% proportion variability) and may have diverse surface coatings and can significantly influence their biological interactions and toxicity. Standardisation can be achieved by using only food-grade E171, removing variation in crystalline form and mixture composition. Similarly, duration of exposure time should be maximised, alongside preference needs to be given to oral administration of TiO₂ NPs rather than other administration routes. Studies have assessed different toxicity endpoints, such as inflammation, oxidative stress, histopathological changes or alterations in enzyme activity, and hence, the choice of endpoints can contribute to variability in reported outcomes. It is important to consider the totality of evidence and recognise that the effects of TiO₂ NPs may depend on specific conditions and contexts.

Epidemiological studies are needed to investigate potential associations between exposure to TiO₂ NPs and adverse health effects in human populations. Long-term observational studies can provide valuable insights into chronic effects on liver and help establish causation. Furthermore, dietary intervention studies in murine models (metabolic associated disease models) based on estimated human intake levels of titanium could clarify the potential risk of E171 in liver health and metabolic disorders. In vivo studies with wider dose ranges via oral administration from different suppliers of E171 (characterising the

physicochemical properties of E171 obtained from local and international vendors) to understand variability of effects can be helpful. While standardising the variables of experimental protocols (such as species, dose, duration of experiment and route of exposure), as may occur for regulatory requirements, may lessen the degree of variability we have noted, these studies are prohibitively expensive. In the absence of this standardisation, we must continue to be cautious with the extrapolation of the results of animal studies to understand the risks of TiO₂ NPs to human liver health. Addressing these challenges requires a collaborative effort within the scientific community. Some of the challenges discussed could be addressed through data sharing and the use of systematic reviews and meta-analyses. Systematic reviews and meta-analyses that aggregate data from multiple studies can help provide a more comprehensive understanding of the potential risks associated with TiO₂ NPs and their impact on liver health. In conclusion, further research is needed to enhance our understanding of the potential risks associated with TiO₂ NPs and to develop guidelines for their safe use in various applications.

CRedit STATEMENT

Jangrez Khan: Conceptualisation; investigation; visualisation; methodology; writing original draft. **Nick Kim:** Supervision; review and editing. **Collette Bromhead:** Supervision; review and editing; resources. **Penelope Truman:** Supervision; review and editing. **Marlena Kruger:** Supervision; review and editing. **Beth Mallard:** Supervision; conceptualisation; visualisation; writing—review and editing.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. Open access publishing facilitated by Massey University, as part of the Wiley–Massey University agreement via the Council of Australian University Librarians. Open access publishing facilitated by Massey University, as part of the Wiley - Massey University agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Khan, J., Kim, N. D., Bromhead, C., Truman, P., Kruger, M. C., & Mallard, B. L. (2024). Hepatotoxicity of titanium dioxide nanoparticles. *Journal of Applied Toxicology*, 1–24. <https://doi.org/10.1002/jat.4626>