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Molecular Mechanism of Nanomaterials Induced Liver Injury: A Review

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Abstract

The unique physicochemical properties inherent to nanoscale materials have unveiled numerous potential applications, spanning beyond the pharmaceutical and medical sectors into various consumer industries like food and cosmetics. Consequently, humans encounter nanomaterials through diverse exposure routes, giving rise to potential health considerations. Noteworthy among these materials are silica and specific metallic nanoparticles, extensively utilized in consumer products, which have garnered substantial attention due to their propensity to accumulate and induce adverse effects in the liver. This review paper aims to provide an exhaustive examination of the molecular mechanisms underpinning nanomaterial-induced hepatotoxicity, drawing insights from both *in vitro* and *in vivo* studies. Primarily, the most frequently observed manifestations of toxicity following the exposure of cells or animal models to various nanomaterials involve the initiation of oxidative stress and inflammation. Additionally, we delve into the existing *in vitro* models employed for evaluating the hepatotoxic effects of nanomaterials, emphasizing the persistent endeavors to advance and bolster the reliability of these models for nanotoxicology research.

INTRODUCTION

In the rapidly advancing field of nanotechnology, the utilization of nanomaterials has become widespread across various industries, promising groundbreaking applications in medicine, electronics, and environmental science. Among the myriad potential benefits, the unique physicochemical properties of nanoparticles (NPs) have enabled remarkable achievements, from targeted drug delivery systems to innovative diagnostic tools. However, this surge in nanomaterial applications has brought forth concerns regarding their safety, particularly in the context of hepatotoxicity^[1]. This comprehensive review aims to delve into the intricate landscape of nanoparticle-induced hepatotoxicity, exploring the diverse range of nanomaterials and their impacts on hepatic function. We will navigate through recent findings on prominent nanomaterials, including silver nanoparticles, carbon nanotubes, quantum dots, and gold nanoparticles, shedding light on the complex mechanisms underlying their hepatotoxic effects^[2-4]. By examining the interplay between nanoparticles and liver cells, such as hepatocytes and Kupffer cells, this review seeks to provide a nuanced understanding of the potential risks associated with nanomaterial exposure.

NPs are classified into four main groups based on structural morphology: organic, inorganic, carbon-based, and composite^[1,5]. Organic nanoparticles, derived from compounds like proteins and lipids, exhibit non-toxic and biodegradable properties, making them suitable for drug delivery, imaging, biosensors, and cancer treatment^[5,6]. Inorganic nanoparticles, including metal-based, metal oxide-based, ceramic, and semiconductor nanoparticles, offer tailored electrical, optical, and magnetic properties for applications in biomedical science, catalysis, and imaging^[5,7]. Quantum dots, semiconductor nanoparticles with size-dependent optoelectronic properties, find applications in electronic and biomedical industries^[8,9]. Carbon-based nanoparticles, such as graphene, fullerenes, and carbon nanotubes, demonstrate unique structural configurations and are utilized in electrical and photonic devices, biomedical sciences, and nanocomposites^[10,11]. Composite nanoparticles integrate different components, leading to unique physical and chemical properties, with three main categories: simple

hybrid, core or shell structured, and multifunctional quantum nanoparticles, applied in electronics, optoelectronics, and biomedical sciences^[12].

To ensure the safety of NPs within the human body, understanding their exposure route is crucial^[13–15]. NPs can be orally exposed through food, drinks, supplements, or nanomedicines, with absorption occurring in organs like the stomach and small intestine. Factors like size, charge, and concentration influence absorption, with NPs under 100 nm diameter taken up directly through endocytosis in the small intestine. Inhalation is another exposure route, with NPs deposited in different regions of the respiratory tract, potentially translocating to other organs. Elimination of NPs from the lungs is complex and depends on physicochemical properties. Dermal exposure, through cosmetics and medications, is facilitated by the skin's permeability to nanoscale particles. Skin penetration varies based on factors like particle size and skin condition. Overall, understanding exposure routes is vital for assessing NP-induced toxicity and ensuring their safe utilization.

Various NPs exert hepatotoxic effects, with silica nanoparticles (SiNPs) showing size-dependent liver injury, synergies with other toxins, and impacts on cholesterol biosynthesis. Nickel oxide nanoparticles (NiO-NPs), tungsten trioxide nanoparticles (WO₃ NPs), and copper oxide nanoparticles (Nano-CuO) induce oxidative stress-related liver damage, apoptosis, and genotoxicity^[2,16–18]. Integrative omics analyses identify key proteins and disrupted metabolic pathways in SiNP-induced hepatotoxicity^[19]. Zinc oxide (ZnO-NPs), titanium dioxide (TiO₂NPs), magnesium oxide (MgO-NPs), aluminum oxide (Al₂O₃NPs), chromium oxide (Cr₂O₃-NPs), and iron oxides (IONPs) exhibit diverse hepatotoxic mechanisms, including oxidative stress, ER stress, inflammation, and disruptions in metabolism^[20–24] (Figure 1 & 2). Carbon nanotubes (CNTs) induce hepatotoxicity through inflammatory responses and oxidative stress, with variations in toxicity based on type and administration method^[4]. Copper sulfide/cadmium sulfide nanoparticles (CuS/CdS-NPs), cobalt nanoparticles, and nanoclay particles also induce oxidative stress-mediated apoptosis and acute hepatotoxicity^[25,26]. Various nanomaterials, such as nanocellulose, polystyrene

nanoparticles, chitosan nanoparticles, hydroxyapatite nanoparticles, quantum dots, and gold nanoparticles, display hepatotoxicity through disrupted redox balance, altered metabolism, necrotic cell death, and impaired mitochondrial function^[8,9,27-29]. The complexity of nanoparticle-induced hepatotoxicity highlights the need for comprehensive assessments and understanding for safe use.

In conclusion, this review not only synthesizes existing knowledge but also highlights critical gaps in understanding nanoparticle-induced hepatotoxicity. By proposing recommendations for future research, we aim to guide the scientific community toward developing safer nanomaterials and fostering responsible practices in nanotechnology. As the field continues to evolve, this exploration into nanotoxicology endeavors to contribute to the ethical and sustainable advancement of nanotechnology.

MAJOR TYPES AND APPLICATIONS OF NANOPARTICLES

Nanoparticles are categorized into four groups based on structural morphology: organic, inorganic, carbon-based, and composite. Some of the most important types of nanoparticles are listed below:

1.1. Organic Nanoparticles:

Organic nanoparticles, derived from compounds like proteins, carbohydrates, lipids, and polymers, encompass micelles, dendrimers, liposomes, nanogels, polymeric NPs, and ferritin ^[6]. Generally non-toxic and biodegradable, they may have a hollow core, such as liposomes, and are sensitive to thermal and electromagnetic radiation. Formed through non-covalent interactions, these labile organic NPs are easily cleared from the body. Nanospheres or nano-capsules, common polymeric forms, collectively referred to as labeled polymorphic NPs, possess properties like a high surface area to volume ratio, stability, inertness, ease of functionalization, and unique optical, electrical, and magnetic behaviors, making them suitable for applications in drug delivery, imaging, biosensors, and cancer treatment^[30].

1.2. Inorganic Nanoparticles:

Inorganic nanoparticles, devoid of carbon atoms, are hydrophilic, non-toxic, and biocompatible, providing high mechanical strength and stability. Precise control over size, shape, and composition allows researchers to design nanoparticles with tailored electrical, optical, and magnetic properties for targeted biomedical applications^[5,7].

1.2.1. Metal-Based Nanoparticles

Metal-based nanoparticles, derived from various metals through disruptive or constructive methods and typically ranging in size from 10 to 100 nm, including aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn), exhibit unique optoelectrical properties due to localized surface plasmon resonance (LSPR) ^[31,32]. Specifically, alkali and noble metals like Cu, Ag, and Au, when utilized in nanoparticle construction, show significant absorption in the visible region of the solar spectrum^[33]. The synthesis of metal nanoparticles with specified facets, sizes, and forms necessitates controlled conditions, and their advanced optical properties make them versatile across various research domains^[5,34]. These nanoparticles, distinguished by their small dimensions and surface properties, including pore size, surface charge, *etc.*, find applications in biomedical science, such as cancer treatment, disease diagnostics, radiation enhancement, drug delivery, and gene transport^[35].

1.2.2. Metal oxide Based Nanoparticles

Metal oxide nanoparticles result from modifying the properties of metal-based nanoparticles. These nano-scale metal oxides find diverse applications in fluorescence, optical sensors, catalysts, biomedicine, gas sensors, and fuel cell anode materials ^[22,36–38]. Various synthesis methods, including inert gas condensation, co-precipitation, and lithography, have been used, but traditional methods often lack control over morphological structure, affecting essential nanomaterial properties^[39,40]

1.2.3. Ceramic nanoparticles

Ceramic nanoparticles, resistant to environmental stresses, form with a solid core through heat or a combination of heat and pressure, incorporating metallic or non-metallic elements^[41,42]. Typically composed of inorganic compounds like silica or

alumina, they may also include metals and metal oxides, yielding diverse nano molecules with varying shapes, sizes, and porosities. Engineered to evade the reticuloendothelial system, ceramic NPs undergo size and surface composition modifications^[43]. Widely used in medical applications, ceramics such as calcium phosphates, alumina, silica iron oxides, carbonates, and titanium dioxide **have been found** ^[44].

Ceramics also play an important role in various applications in photocatalysis, dye photodegradation, imaging, and catalysis ^[45]. Researchers aim to develop advanced ceramics with minimal cytotoxicity and enhanced biocompatibility, addressing challenges through innovative strategies that integrate ceramic nanoparticles with biocompatible materials, considering characteristics like shape, size, and physicochemical attributes ^[46].

1.2.4. **Lipid-Based** Nanoparticles

Lipid-Based Nanoparticles (LBNPs), typically 10-100 nm in diameter, consist of a lipid core surrounded by lipophilic molecules, finding applications in oncology and biomedicine ^[47]. Liposomes, a key type of LBNP, use a phospholipid bilayer for enhanced drug solubility and stability, accommodating both hydrophobic and hydrophilic molecules. Incorporating cholesterol improves stability, decreases fluidity, and enhances permeability for hydrophobic drugs in liposomal formulations ^[48]. Solid Lipid Nanoparticles (SLNs), sized between 50-1000 nm, and composed of physiological lipids in a solid state, offer a compelling alternative for drug delivery, featuring a matrix of mono-, di-, or triglycerides, fatty acids, and complex glyceride mixtures, with stability ensured by surfactants or polymers^[49].

1.2.5. Semiconductor Nanoparticles

Semiconductor nanoparticles, possessing hybrid characteristics of metals and nonmetals, have garnered attention for their versatility in diverse applications ^[50,51]. Their crucial broad bandgap, adjustable by researchers, makes them valuable in photocatalysis, photo optics, and electronic devices ^[52]. Additionally, their nano-scale dimensions provide benefits such as increased surface area-to-volume ratio, enhanced

quantum confinement effects, and improved catalytic activity, contributing to exceptional performance in various applications [53].

1.3. Quantum Dots

Quantum dots (QDs), semiconductor nanoparticles with size- and composition-dependent optoelectronic properties (1.5 to 10.0 nm), play a significant role in the electronic and biomedical industries^[8]. Their success is attributed to superior features like ²⁵ photostability, size-dependent optical properties, high extinction coefficient, brightness, and a large Stokes shift, overcoming limitations of organic dyes. QDs, due to their ultrasmall size, are well-suited for imaging and biosensing applications. They facilitate the development of multimodal/multifunctional probes with increased surface area for optical trackability *in vitro* and *in vivo*, designed to detect pH, metal ions, DNA, and enzyme activity, and deliver various therapeutics^[8].

1.4. Carbon-based nanoparticles:

Carbon-based nanoparticles encompass five main materials: carbon nanotubes, graphene, fullerenes, carbon nanofiber, and carbon black, each with unique structural configurations and diverse applications in nanotechnology.

1.4.1. Graphene

Graphene, a two-dimensional ⁵ carbon allotrope, is a single layer of carbon atoms arranged in a hexagonal lattice with exceptional properties, such as elasticity, mechanical strength, and unparalleled thermal and electrical conductivity. Synthesized in the laboratory, it forms a 1nm-wide honeycomb lattice, exhibiting semiconductor properties without an effective mass and zero band gap. Graphene demonstrates an ambipolar electric field effect, with a ²² breaking strength of 42 Nm⁻¹ and a Young's modulus of approximately 1.0, making it the strongest material ever tested. These attributes position graphene as a promising material for electrical and photonic devices, sensing platforms, and clean energy applications^[5].

1.4.2. Fullerene

Fullerenes, a molecular form of carbon allotrope, consists of C_n clusters (n>20) arranged on a spherical surface with carbon atoms at pentagon and hexagon vertices^[54]. The

extensively studied C60 fullerene, composed of 60 carbon atoms, is highly symmetric and spherical, with a 0.7nm diameter and sp² hybridized carbon atoms. Exhibiting exceptional symmetry and stability, fullerenes have 20 tripled axes, 12 fivefold axes, and 30 twofold axes^[54]. These unique properties position fullerenes as promising nanoparticles widely utilized in biomedical sciences, acting as inhibitors for human immunodeficiency virus, contrast agents for magnetic resonance imaging, and sensitizers for photodynamic therapy ^[5,55].

1.4.3. Carbon nanotubes

Carbon nanotubes (CNTs), unique in carbon-based nanomaterials, possess versatile characteristics like length, diameter, chirality, and layer number, showcasing exceptional properties and widespread applications. Composed of graphite, CNTs, typically with at least two layers and an outer diameter ranging from 3 nm to 30 nm, are divided into two categories: single-walled nanotubes (SWCNTs) and multi-walled nanotubes (MWCNTs). SWCNTs, with a diameter of around 1 nm, exhibit high electrical conductivity, mechanical strength, and thermal conductivity due to their nearly one-dimensional structure, indicated by a length-to-diameter ratio of approximately 1000^[56]. MWCNTs, robust cylindrical structures with a minimum diameter of 100 nm, demonstrate resilience and diverse structures rooted in graphene sheets, with an interlayer distance resembling that in graphite, about 3.3 Å. The initial proposal for gram-scale synthesis of double-walled carbon nanotubes (DWCNTs) in 2003 involved chemical vapor deposition (CVD), selectively reducing oxide solid solutions in methane and hydrogen^[10,56–58]. Applications of CNTs include bicables, AFM tips, hydrogen storage, electrochemical electrodes, nanocomposites, field emission displays, and diverse electrical devices^[59].

1.5. Composite nanoparticles

Composite nanoparticles are produced *via* the integration of two or more different components. The components bear different properties at the nanoscale level. This integration of diverse components eliminates the limitation of individual components which enables researchers to produce nanomaterials with specific properties and uses.

These NPs exhibit unique physical and chemical properties and each component has strong mutual coupling effects on the other. The chemical properties of composite nanoparticles depend on their composition and structure. The mutual coupling effect between the components of composite NPs can lead to changes in the chemical properties of composite NPs^[12]. Composite NPs are used in a variety of applications including electronics, optoelectronics, and biomedical sciences^[60].

Composite nanoparticles can be classified into three main categories based on their structural features:

1.5.1. Simple Hybrid NPs

These types of composite NPs formed by combining two or more components without a specific structural hierarchy. They exhibit unique properties due to the combination of different materials^[61].

1.5.2. Core or Shell Structured Composite NPs

These NPs are made up of two different regions: an inner core region and an outer shell. These two regions of NPs are composed of two or more different materials. The core and shell structure influences the properties of the nanoparticles, such as electromagnetic wave attenuation capacity, etc^[62].

1.5.3. Multifunctional Quantum NPs

These NPs have multiple functionalities, such as magneto-optical, and electrochemical properties. The specific structure of Multifunctional Quantum Composite NP is used in applications like biosensing, bioassays, catalysis, and separations^[12].

EXPOSURE ROUTES OF NANOPARTICLES

A myriad number of nanoparticles are manufactured from diverse materials to serve a multitude of purposes, it is crucial to ensure the unswerving safety of these particles within the human body. To understand the degree and mechanism of nanoparticle-induced toxicity, it is essential to understand their route of exposure, toxicological profile, and fate in the human body. The route of exposure also acts as a crucial factor

in deciding the potential toxicity of NPs. The potential routes of NP exposure are as follows:

1.1. Oral Exposure

Oral Exposure of NPs occurs following intake of food, drinks, or additives and supplements containing NPs, swallowing of inhaled NPs, or oral administration of nanomedicines or nano-formulations. These particles are then passed through the following organs **esophagus**, stomach, small intestine, and large intestine, and are readily absorbed in the stomach epithelial cells [13,14,63].

However, the absorption rate of NPs depends on multiple factors such as shape and size, concentration, pH of the medium, *etc.* The size and charge of the NPs also influence the absorption rate; positively charged NPs were captured through negatively charged mucus, whereas, negatively charged nano-molecules easily entered the mucus layer. Particle size also plays a crucial role because larger NPs required more for ingestion as well as digestion^[64,65]. It has been observed that NPs lower than 100 nm diameter, are directly taken up by endocytosis through regular epithelial cells of the small intestine ^[66,67] Absorption can also occur through **epithelial cells of Peyer's patches in the gut-associated lymphoid tissue**. Some other research studies proposed that oral intake of NPs could be absorbed in the gastrointestinal tract, from where the particles can transigrate to the liver and spleen *via* lymph nodes.

1.2. Inhalation

Nanoparticles have been observed to exert **their** effect on human health, primarily *via* dermal contact or inhalation. The NPs inhaled during production or usage, get deposited all over the respiratory tract and the smaller particles penetrate the lungs where they accumulate in the alveolar regions. The larger NPs with diameters ranging from 5-30 μm usually reside in the nasopharyngeal region and the smaller particles, with diameters ranging from 1-5 μm tend to deposit in the tracheobronchial region. The smallest NPs (0.1–1 μm) deposited over the alveolar region^[68,69]. The particles smaller than 10mm are primarily absorbed inside the lung and may undergo translocation to various parts of the body including **the** kidney. Insoluble particles accumulated in the

lung have the potential to trigger diverse local toxicological reactions. The smaller NPs easily translocated compared to the bigger ones, and after reaching the lung they can remain there for years and can make their way into the circulatory or lymphatic system and subsequently disseminate into other organs like the liver, spleen, and kidneys^[15].

The elimination procedure of NPs is very complex and lengthy and depends on its physicochemical properties. The larger particles which are deposited at the extra-thoracic and intrathoracic bifurcation, have been trapped in the mucus layer and transported through the mucociliary escalator into the pharyngeal region. These mucus-laden NPs are then swallowed and enter into the gastrointestinal tract for further processing. The smaller particles in bronchioles and alveoli undergo mucus-associated transport and are then phagocytosed by alveolar macrophage. However, if these strategies are unable to reduce the toxicity, the lung defense system becomes stronger and eventually causes lung tissue damage^[11,26,70]

1.3. Dermal exposure

Skin, the largest organ and primary protective barrier of the human body acts as the easiest route of NP entrance. ¹⁰ The skin is divided into three layers: epidermis, dermis, and hypodermis. While the epidermis effectively prevents the entry of micrometer-sized particles, but less effective as a barrier for particles in the nanoscale range. Dermal exposure to nanoparticles is unavoidable with the use of various cosmetics and medications. Several experimental investigations examined the feasibility of nanoparticle penetration through the skin barrier and reported that NPs are unable to traverse the skin whereas, contrasting findings from other studies, specifically those focused on metal NPs such as iron NPs, reported that they can successfully penetrate through hair follicles and ultimately reached to the basal and spinous layers^[71,72]. The epidermal entry of NPs is influenced by a variety of factors such as exposure medium, medium pH, temperature, etc^[13,14,63].

¹⁰ The existing evidence suggests that NPs with a diameter of about 4 nm can permeate intact skin whereas, when the size grows up to 45 nm, NPs can only permeate *via* ¹¹ impaired or injured skin^[73]. Beneath the dermal layer rich with blood vessels,

macrophages, lymph vessels, dendritic cells, and nerve endings. Consequently, particles absorbed beneath distinct layers of the skin undergo swift transport within diverse circulatory systems^[1].

NPS MEDIATED HEPATOTOXICITY

1.1. Silica nanoparticles

A series of investigations revealed that the administration of silica nanoparticles with smaller diameters (30nm) exhibited more liver injury or lethality compared to larger ones (1000nm) ^[2,74,75]. Suggesting an inverse correlation between the silica nanoparticle size and hepatotoxicity. Also in combinatorial toxicity assessment, SP30 (30nm), the smallest NPs was found to synergize the other known chemical liver toxins (carbon tetrachloride, paraquat, cisplatin) in causing hepatic damage ^[75]. In another study, increased biodistribution with reduced urinary excretion was observed for lower aspect ratio (AR) of mesoporous silicon nanoparticles ^[76]. In an *in vitro* study when four amorphous SiNPs with different surface areas were applied on HepG2 cells, a clear perturbation in cholesterol biosynthesis was observed. Increased cholesterol biosynthesis was found to be directly proportional to the increased surface area, which might have an impact on steroidogenesis and bile formation ^[19]. In a metabolomic study, the same group demonstrated amorphous SiNPs mediated depletion of glutathione, NADPH oxidase mediated ROS production, and alterations in antioxidant profile indicating perturbation of glutathione metabolism and glutathione pool in hepatocytes ^[77].

In a dose and time-dependent manner, mesoporous SiNPs (MSN) caused cytotoxicity in L-02 cells. In NLRP3 knockout mice and caspase-1 knockout mice model, MSN-promoted inflammation and hepatotoxicity were found to be abolished compared to the normal mice, suggesting mSiNPs mediated ROS overproduction followed by activated NOD-like receptor protein 3 (NLRP3) inflammasome, resulting into pyroptosis through caspase-1 activation ^[78]. Rat exposed to silica NPs compared to control exhibited altered liver biochemical parameters such as elevated levels of LDL, ALP, AST, and ALT along

with prolactin, iron, phosphorus, and potassium concentration. Histological modifications include Hydropic degeneration, Karyopyknosis, Sinusoidal dilatation, Hyperplasia of Kupffer cells, and infiltration of inflammatory cells with lowered liver index. Also negatively affects the expression of phase I and phase II drug metabolizing and drug transporter genes (*slc2a1*, *cyp4a12*, *ephx2*, *nat2*) [79].

Kupffer cells (Kc) are well-known resident macrophages of the liver, contributing to the maintenance of liver normal physiological activity and homeostasis. Excessive accumulation of ROS and simultaneous release of bioactive mediators (H_2O_2 , NO, and $TNF\alpha$) indicates SiO_2 NPs mediated activation and hyperplasia of KCs. BRL cells exhibited reduced viability, and structural alterations along with elevated levels of marker enzymes (LDH, AST) when co-cultured (contactless) with SiNPs activated KCs, clearly suggesting that KCs activated by SiO_2 NPs can cause liver injury via the release of H_2O_2 , NO, and $TNF\alpha$. In addition to that, infiltration of inflammatory cells and subsequent increase of $TNF\alpha$, monocyte, lymphocytes, and neutrophils in the liver can be correlated with SiNPs activated KCs mediated inflammation in the liver [80].

Analysis of 1H nuclear magnetic resonance (1H NMR) results, unveiled lipid metabolism disorder in rats receiving intratracheal instillation of SiNPs causing hepatotoxicity in a dose-dependent manner. Biochemical analysis showed a significant increase in ALT, AST, TG, and LDL-C levels but a decrease in HDL-C levels in the treated group. Ten metabolic pathways were affected due to treatment, including the metabolism of amino acids (glutamate, cysteine, aspartate), purines, and glucose-alanine cycle that resulted in the production of 11 different metabolites compared to control [81].

Autophagy-mediated liver toxicity involves ALR (autophagic lysosomal reformation) an event where anomalous autophagy fails to terminate, which results in a persistent accumulation of enlarged autolysosomes. Mouse hepatocytes on exposure to SiO_2 NPs prevent conversion of PI(4)P to PI(4,5)P₂ on enlarged autolysosomal membrane due to loss of PIP5K1B, also clathrin fails to be recruited, leading to suppression of ALR and resulted into enlarged autolysosomes [82]. The molecular mechanism behind SiNp-

induced autophagosome synthesis, accumulation, and autophagic dysfunction was worked out on L-02 cells. When treated with different concentrations of SiNPs, readily get internalized and induce ROS production, which in turn **causes** ER stress and UPR. Upregulated expressions of ATF4 and DDIT3 indicate involvement of EIF2AK3 and ATF6 pathway but not ERN1-XBP1 pathway. ATF4 and DDIT3 then transcriptionally upregulate expressions of LC3B and ATG12 (autophagic genes) that **result in** autophagosome formation^[83]. In HepG2 cells accumulation of amorphous SiNPs in mitochondria leads to excessive ROS generation that in turn **triggers** autophagy and autophagic cell death in hepatocytes *via* the PI3K/ Akt/ mTOR pathway ^[84].

Overexpression of p53, bax, **and** caspase-3 in contrast to bcl-2 downregulation along with ROS generation in HepG2 cells insulted with SiNPs suggests activation of cell cycle check point genes and apoptotic pathway in accordance to cytotoxicity due to oxidative stress. Restoration of cell viability with **an altered** apoptotic marker profile was observed in the same cell when co-treated with vitamin C, a ROS scavenger ^[85]. Amorphous SiNPs exposure to human cells (HL-7702) and rat cells (BRL-3A) showed elevated expression of p53, Bax, cleaved caspase-3, and negative expression of Bcl-2 and caspase-3 Levels, with increased ROS generation and decreased GSH level indicating oxidative **stress**-mediated cytotoxicity that leads to apoptotic activation *via* p53/casp-3/Bax/Bcl-2 pathway. Human liver cells exhibited more sensitivity than rat liver cells ^[86].

Compared to normal mice, SiNPs exhibited more severe effects **in the** liver of metabolic syndrome mice though improved insulin resistance. It has been established that SiNP exposure can accelerate liver damage in metabolic syndrome mice following deposition to mitochondria which **results in** mitochondrial injury and overproduction of ROS. That aggravated liver fibrosis (higher collagen deposition), hepatic ballooning, DNA damage (genotoxicity), **and** infiltration of inflammatory cells ^[87]. A recent study reveals SiNPs induced hepatotoxicity *via* perturbing mitochondrial quality control (MQC) process, promoting excessive mitochondrial fission (**DRP1, FIS1, and MFN2 were up-regulated under SiNPs exposure, but MFN1 was down-regulated**), mitophagy disorder

(PINK/Parkin signaling, up-regulated PINK1 and *p*-Parkin, as well as an enhanced conversion of LC3B-I to LC3B-II) and downregulating mitochondrial biogenesis (inhibited mitochondrial biogenesis *via* PGC1 α -NRF1-TFAM signaling, decline PGC1 α , NRF1 and TFAM), leading to mitochondrial dysfunction followed by hepatocyte damage and liver biotoxicity [88]. From the above findings, it can be speculated that mitochondrial injury & instability in hepatocytes due to SiNP exposure resulted in liver oxidative stress.

Recent *in vitro* as well as *in vivo* investigation results indicate silicon NP insult can trigger LDH, ALT, and AST in serum concentration owing to hepatic damage. Compromised antioxidant enzyme profile (CAT, SOD, and GPx) with elevated levels of oxidative stress markers (NO, MDA, PCO, and H₂O₂) and MDA levels are engaged in hepatic ROS production [89]. Altered hepatic metabolism is observed in both free fatty acid (FFA)- treated L-O2 cells and ApoE^{-/-} mice model receiving SiNps treatment. Increased fatty acid biosynthesis, lipid deposition, liver TC/TG index along with decreased β -oxidation and lipid efflux resulting into perturbed lipid metabolism can be corroborated with the induction of oxidative stress-related liver injuries, may help the acceleration of liver diseases like metabolic associated fatty liver disease (MAFLD) [90]. More over-upregulated expressions of pro-apoptotic genes (Bax, p53, Caspase-9/3) and downregulated anti-apoptotic genes Bcl-2 along with histopathological alterations of the liver such as sinusoidal dilatation, Kupffer cell hyperplasia, infiltration of inflammatory cells strongly indicates SiNPs induced hepatic toxicity *via* ROS-activated caspase signaling pathway, leading to induction of apoptosis in the liver [89]. Through integrative proteomic and metabolomic analyses, Zhu *et al* identified key proteins (RPL3, HSP90AA1, SOD, PGK1, GOT1, PNP) indicative of abnormal protein synthesis, oxidative stress, and metabolic dysfunction in SiNP-induced hepatotoxicity [91]. Metabolomic data revealed disruptions in vital metabolites (glucose, alanine, GSH, CTP, ATP). Bioinformatic analysis highlighted disturbances in glucose and amino acid metabolism, suggesting potential exacerbation of oxidative stress and liver injury. Key proteins associated with SiNP-induced hepatotoxicity

include SOD, TKT, PGM1, GOT1, PNP, and NME2^[91]. This study underscores the power of integrative omics analyses for nanoparticle toxicity assessments. Follow table 1 for a comprehensive account.

1.2. Metal Oxides nanoparticles

Consult table 2 to table 10 for a comprehensive account of different metal oxide-induced hepatotoxicity.

1.2.1. Nickel oxides nanoparticles

The findings of several stress assays, liver function tests, and histopathology analyses make it abundantly evident that rats given NiO-NPs experience oxidative stress and oxidative stress-related liver damage ^[92,93]. Chang *et al* demonstrated that the liver cells of rats injected with NiO underwent ER stress and that this brought about the induction of apoptosis *via* many routes, including the PERK/eIF-2 α , IRE-1 α /XBP-1S, and caspase-12/-9/-3 pathways ^[16]. A different investigation using a comparable experimental design found that the NF- κ B signaling pathway is associated with hepatotoxicity ^[94].

In the HepG2 cells model, NiONPs caused cytotoxicity through ROS production and Bax/Bcl-2 pathway-mediated apoptotic induction. Also treated cells exhibited micronuclei formation, chromatin condensation, and DNA damage suggesting NiONPs mediated genotoxicity ^[95]. NiO was additionally found to induce hypoxic stress in the same human liver cells in a concentration-dependent manner, as evidenced by the activation of hallmark candidate genes, hypoxia-inducible transcription factor-1 α (HIF-1 α), and miR-210 microRNA and decreased levels of ribosome biogenesis. Nitric oxide (NO) levels that were too high caused Ca⁺⁺ influx, which in turn led to mitochondrial instability and oxidative stress, further encouraging lysosomal degradation in connection with autophagic processes. Subsequently led to the development of apoptosis *via* the p53 and MAPKAPK-2 signaling pathways ^[96]. Rat liver and HepG2 cells under Nano-NiO exposure resulted in hepatic fibrosis. Upregulation of TGF- β 1, Smad2, Smad3, alpha-smooth muscle actin (α -SMA), matrix metalloproteinase 9 (MMP9), tissue inhibitors of metalloproteinase1 (TIMP1) but simultaneously downregulation of E-cadherin and Smad7 in both models can be corroborated with

hepatic fibrosis *via* activation of TGF- β 1/Smad pathway, epithelial-mesenchymal transition (EMT), reformation and deposition of extracellular matrix (ECM) [97]. A recent study reported NiNps-mediated hepatic injury following hepatic inflammatory response, ER stress, abnormal lipid metabolism that leads to hepatocyte apoptosis [98]. NiNPs exposure (15-45 mg/kg) in rats induced dose-dependent liver dysfunction, histological injuries, and oxidative stress. Elevated NF- κ B, nitrate stress markers, and inflammatory and apoptotic mediators were observed. The study highlights Ni NPs-induced hepatotoxicity, crucial for health risk assessment[99].

1.2.2. Tungsten trioxide nanoparticles

WO₃ nanorods of varying lengths have been shown to cause hepatotoxicity in mice when given intraperitoneally. This effect is evident in the form of hepatocytic lesions, which include cellular edema, nuclear pyknosis in most hepatocytes, cytoplasmic vacuolation, and hydropic degeneration in hepatocytes surrounding the central vein. Additionally, liver function is impaired, as evidenced by elevated levels of serum ALT and AST, which are caused by oxidative stress (increased intracellular ROS, significant reduction in GSH and SOD activity), as well as an inflammatory response (increased NF- κ B, TNF- α , IFN- γ , and IL-4). Shorter nanorods showed greater toxicity than longer nanorods in terms of severity. Adversity of WO₃ nanorod was decreased by melatonin administration[17].

1.2.3. Copper oxide nanoparticles

Transmission Electron Microscope (TEM) investigation has confirmed the accumulation and distribution of CuONPs in rat liver tissue after oral administration. This would indicate that CuONPs can be easily absorbed through the intestinal wall and transported to the liver *via* blood. Serum levels of bilirubin that are high, heightened catalase and SOD activity, and altered glutathione metabolism enzyme profiles (GR, GPx, and GST), all strongly suggest that NPs exacerbated oxidative stress-related liver damage [100]. The primary marker of hepatic injury is an increase in vital enzymes such as serum Alanine aminotransferase (ALT), and serum Aspartate aminotransferase (AST) in the liver. CuONP-treated Wister rats have been shown to have

histopathological changes, such as pyknotic, pleomorphic nuclei, binucleated hepatocytes with an increased population of apoptotic cells, with elevated levels of AST, ALT, and decreased levels of albumin in serum^[18]. Mice receiving both chemically and biologically synthesized CuO-NPs (CNPs and BNPs), but mostly BNPs, showed distinct histopathological, biochemical, and apoptotic changes. Various types of histopathological alterations in hepatic tissues against **their normal** functioning range from hydropic degeneration and vacuolization to cell necrosis, loss of plasma membrane, more eosinophilic cytoplasm, karyorrhexis and complete loss of nucleus in few cells, activated Kupffer cells, lymphocytic infiltration around necrotized cells and congestion in sinusoids. Biochemical examination showed elevated levels of serum ALT and AST. Increased expression pattern of P⁵³, Casp-3 immunoreactivity suggested induction of apoptosis due to CuO toxicity in liver cells^[101]. A comparable study has reported additional architectural abnormalities, such as ER swelling with lower count, increased intracellular space, fat accumulation, and cellular shrinkage related to the distribution of Nano-CuO in the liver. These discrepancies have been shown to affect hepatocyte growth, metabolism, and viability in both *in vitro* and *in vivo* investigations. JNK, PERK, CHOP, ATF4, eIF2 α , IRE1, Calpain, GRP78, ATF6, Bax, Caspase-3, and Caspase-12 have all been shown to have upregulated expressions in treatment group while Bcl-2 expression level gets diminished, is consistent with ROS-mediated oxidative stress-induced activation of the ER-stress pathway, that triggered apoptosis in liver tissue cells^[102]. The liver of adult rats treated with CNPs (chemically **synthesized** CuO NPs) showed dose-dependent genotoxicity (DNA tailing), an enhanced oxidative stress response (lipid peroxidation), and histopathological changes (dilation and congestion of sinusoids) in contrast to GNP (green **synthesized** CuO NPs)^[103]. Mild to severe deleterious alterations in hepatic tissues including disorganized hepatic rays, dilated sinusoids with congestion, hepatocytic necrosis, glycogen breakdown, hemosiderosis, steatosis, hyperplasia **of the bile** duct and fibrous tissue proliferation, anti-inflammatory cell infiltration with caspase 3 **immunoreactivity were also** observed **against the administration** of nano-CuO in **dose-dependent** manner^[104].

1.2.4. Zinc Oxide nanoparticles

In a study, Pasupuleti *et al* reported that when SD rats were orally given nano-sized and micro-sized ZnO (5-2000mg/kg), compared to micro-sized zinc oxide, nano-size zinc oxide exhibited an inverse dose-dependent increase in AST and ALT, that means nano-sized ZnO have shown higher toxicity at lower doses^[20]. Suggesting liver tissue assault and degeneration. Contrary to this result, Yang *et al* demonstrated dose-dependent nanotoxicity of ZnO in mice models ^[105]. A significant decrease in antioxidant (GSH) level causes an imbalance between oxidants and antioxidants, resulting in oxidative stress in the liver. Elevated expressions of transcription factor (xbp-1), ER chaperons (grp78, grp94, pdi-3), and phosphorylation of PERK and eIF2 α in association to ER swelling and damage in hepatocytes strongly indicate ER stress. Upregulated expressions of proapoptotic genes (bax, chop), initiator caspase (Casp-9,12), effector caspase (casp-3), and subsequent diminished expression of bcl2, phosphorylation, and activation of JNK and CHOP/GADD153 strongly suggested ER stress-mediated opening of apoptotic pathways in liver tissue treated with nano-ZnO. Exposure to ZnONPs produces histological and histochemical modifications in liver tissues that may affect normal functioning. Degenerative liver cells exhibited nuclear changes (nuclear membrane irregularity, binucleation, nuclear vesiculation, anisokaryosis, and karyolysis), cytoplasmic changes (cytoplasmic vacuolation with parietal cytoplasmic swelling), and glycogen depletion followed by necrosis under ZnO insult. Inflammatory signs were sinusoidal dilation following Kupffer cell activation and enlargement, infiltration of inflammatory cells at lobular and portal triad ^[106]. ZnO-NPs-induced inflammatory liver injury *via* the production of inflammatory mediators (NO, TNF-, IL-6, CRP, IgG) has been documented ^[107]. Human liver cell HepG2 in response to short exposure to ZnO exhibited oxidative stress-mediated cytotoxic effects leading to LDH leakage, DNA damage, reduction in MMP, and increment in the ratio of proapoptotic/antiapoptotic proteins that lead to activation of mitochondrial apoptotic pathway. In addition to that ZnO was found to induce the phosphorylation of JNK, P38,

and P53^{ser15} without any significant changes in their expression level [108]. Above mentioned hepatic histopathological and immunohistochemical alterations along with oxidative stress are found to be promoted via modulation of JNK/p38MAPK and the STAT-3 signaling pathways [109]. A separate investigation in HepG2 cells revealed that ZnONPs override the toxic effects of ZnO (zinc oxide), exhibiting more hepatocyte inactivation, oxidative stress, mitochondrial damage, elevated intracellular calcium load along with weaker antioxidant level, and severe histopathological distortions. The expression pattern of differentially expressed genes (DEGs) and their transcripts are more for ZnONPs [110]. A recent study confirms the cytotoxic and genotoxic potentiality of ZnONPs in HepG2 cells in 2D and 3D culture after 24 h of exposure [111]. In dogs overused ZnONPs enhanced zinc accumulation in the liver with elevated serum liver indexes along with ROS generation and altered mitochondrial function. Strikingly ZnONPs attenuated apoptosis via the cytochrome c pathway instead, it induced autophagy through activating the mTOR/ATG5 pathway. Also involved in the disruption of the intestinal microbiome and 81 Liver metabolites [112]. ZnO NPs induce crosstalk between protective autophagy and pyroptosis in hepatocytes. TFEB-mediated regulation influences ZnO NP-induced pyroptosis, with TFEB knockout exacerbating and overexpression alleviating it. TRAF-6 is identified in TFEB-mediated global regulation [113]. TFEB-regulated autophagy and lysosome prevent ZnO NPs-induced hepatocyte pyroptosis, providing insights for risk assessment and therapeutic strategies [113]. ZnO NPs also widely used in various applications, induce oxidative stress, leading to NLRP3-ASC-Caspase-1 complex assembly and pyroptosis in rat liver and HepG2 cells [114]. Inhibiting oxidative stress protects against ZnONPs-induced pyroptosis in hepatocytes, revealing a novel mechanism and potential clinical treatment strategies [114].

1.2.5. Titanium dioxide nanoparticles

Several major effects and molecular mechanisms underlying hepatotoxicity due to TiO₂NP exposure have been reported in both *in vitro* and *in vivo* studies. Titanium dioxide exists in different commercially available forms. The natural one is an

agglomerated, rod-shaped rutile form and the other is a glomerated metastable form, the anatase. Chen *et al*, in a study proved that both forms can significantly activate inflammatory signaling pathways like mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF- κ B) in HepG2 cells with reduced cell viability and ultrastructural alterations, though rutile form has more cytotoxic effect^[115]. In 80 CD-1 (ICR) mice, intragastric administration of TiO₂NPs resulted in increased expressions of Toll-like receptors (TLR2 & TLR4) and inflammation-related genes (IKK1, IKK2, NF- κ B, NF-kBP52, NF-kBP65, TNF- α , NIK) with decreased expressions of I κ B and Il-2 indicating TLRs/NIK/I κ B kinase/NF- κ B/TNF- α signaling pathway mediated inflammation in liver. At higher doses significant changes in liver coefficient, biochemical parameters [ALT, AST, ALP, LDH, PChE (pseudocholinesterase), LAP (leucine acid peptide)] along with mitochondrial swelling, apoptotic body formation, chromatin condensation, inflammatory cell infiltration suggests liver tissue injury caused by inflammation that in turn trigger activation of apoptosis^[116]. The same group showed that TiO₂NPs insult leads to ROS accumulation, over-expression of cytochrome p450 1A, and suppressed expression of stress-related genes (SOD, CAT, GSH-Px, MT, HSP70, GST), and NPs detoxifying/metabolizing genes^[117]. Other investigation result shows that TiO₂NP ingestion at higher doses for longer periods leads to Kupffer cells hypertrophy, hydropic degeneration and vacuolization in hepatocytes, necrosis around the central vein followed by edema, infiltration of inflammatory cells along reduced antioxidant enzymes. Elevated levels of liver enzymes, higher lipid peroxidation, and upregulated expressions of inflammatory mediators (TNF α and NF-K β) suggest hepatic damage due to oxidative stress and inflammation^[118].

Different spectral analyses and gel electrophoresis results of *in vivo* experiments unveil that liver DNA is a prime target of TiO₂NPs. In liver DNA, anatase form get accumulates either by inserting itself between base pairs or directly binding to 3 oxygen or nitrogen atoms [Ti-O(N)=1.87Å] and 2 phosphorous atoms (Ti-P=2.38Å) of nucleotide, affecting the configuration of DNA secondary structure. DNA laddering in gel slab at a higher dose of 150 mg/kg can be corroborated with liver DNA cleavage by

NPs [119]. *In vitro* study with HepG2 cells also exhibited oxidative stress-induced DNA damage for both rutile and anatase forms. Elevated expression level of p53 and subsequent upregulated expression pattern of downstream DNA damage responsive genes (p21, mdm2, gadd45α) confirms the TiO₂NPs mediated genotoxicity in hepatocytes^[120]. Gene expression analysis and genotoxicity assessment demonstrated similar results, that TiO₂NPs promote oxidization of nucleotides which results in DNA strand break (DNA damage). Also disturbs the metabolic homeostasis of the liver through oxidative and stress-related impairment of glucose, lipid, and xenobiotic metabolism^[121].

When primary hepatocytes were given exposure to rutile, anatase, and P25 (mixture of rutile & anatase) NPs, all three significantly exhibited hepatotoxicity. The Mitochondrial morphology and dynamics get compromised due to the downregulation of the fusion process, which leads to mitochondrial fragmentation in hepatocytes. Over production of ROS and subsequent loss of MnSOD enzyme activity and reduced MMP leads to oxidative stress that hampers the normal functionality of liver cells including biosynthesis of urea and albumin^[122]. In a remarkable *in vitro* as well as *in vivo* experimentation Sha *et al* Click or tap here to enter text. have proven that liver cells already in oxidative stress condition exhibit more susceptibility towards nano-TiO₂ mediated cytotoxicity^[123]. In contrast to G0/G1 phase arrest under only NM exposure, BRL-3A cells with prior oxidative stress conditions exhibited very fast G0/G1 phase to S transition, G2/M arrest with elevated cell death ratios. Increased expression levels of liver marker enzymes (ALT, AST, ALP, LDH) under the same experimental regime in an *in vivo* study indicated liver damage with prominent histopathological perturbation. Micro-TiO₂ didn't show such effects both in cells and rat liver. Again, in different studies orally administered thymol and tiron were seen to ameliorate TiO₂NPs mediated LPO, oxidative stress, non-enzymatic and enzymatic alterations of antioxidant levels, augmentation of proapoptotic and downregulation of antiapoptotic genes along with biochemical and histopathological changes in liver tissue. Supporting hepatic injury by TiO₂NPs is mediated by oxidative stress and apoptosis^[124,125].

In a dose-dependent manner TiO₂NPs treated rats show an increment in diversity and abundance of gut microbiota (*Firmicutes*, *Bacteroidetes*, *Tenericutes*, *Proteobacteria*, etc.) that has been found to produce a significant quantity of lipopolysaccharides (LPS) and increased number of *Lactobacillus reuteri* but not *Romboutsia* in feces. On the contrary, it produces mitochondrial swelling, and an imbalance in oxidation/antioxidation status with the generation of altered metabolites (Glutamate, glutamine, and glutathione) in connection to energy-related metabolic disorders. Therefore, it can be predicted from the results that the indirect pathway of the gut-liver axis may play an important role, in connecting gut microbiota and liver metabolism. Subsequent investigation confirms that the gut microbiota under oxidative stress led to lipid metabolism disorders (glycerophospholipid and phosphatidylcholines) and caused liver toxicity via the gut-liver axis [126,127].

1.2.6. Mg-nano nanoparticles

In a recent experiment, researchers have tried to verify the hepatotoxic potentiality of Mg-nano in combination with valproate (anticonvulsant drug) and PTZ (pentylentetrazole- used to induce convulsion mouse model) using 3D liver organoid and rat model. In the *in vitro* model the prepared suspension carrying Mg-nano decreased the production of ATP and increased ROS generation and super oxide production while *in vivo* result showed a significant increase of ALT, AST in serum but without any change in albumin or globulin concentration, suggesting Mg-nano as well as Valporate both can induce hepatotoxicity [21].

1.2.7. Aluminium oxide nanoparticles

Aluminum oxide nanoparticles (Al₂O₃-NPs) pose hepatotoxic effects on chicken embryos and cell cultures, inducing histological abnormalities, elevating tissue damage markers, causing oxidative stress, and impacting antioxidant enzymes[128]. Additionally, Al₂O₃-NPs affect red blood cells, liver metabolism, and stress response gene expression. The study reveals dose-dependent ROS generation, cytotoxic responses, and potentiating effects on TNF- α -induced apoptosis. Inhibition of p38 MAPK and JNK

pathways modulates Al₂O₃-NPs-induced apoptosis in HepG2 cells, highlighting novel mechanisms and potential prevention strategies^[128].

1.2.8. Chromium oxide nanoparticles

The investigated liver function biomarkers (ALT, AST, ALP, γ GT, total bilirubin levels) get elevated in a dose and exposure time-dependent fashion in rats after orally consuming Cr₂O₃-NPs. Routine histological examination clearly showed moderate to severe architectural damage including liver cell degeneration, Kupffer cell hyperplasia, parenchymal distortions, dilated central vein, and hemorrhage for both low and high doses, indicating the role of chromium oxide-NPs in liver toxicity ^[23].

1.2.9. Iron oxides nanoparticles

The bioavailability of nano iron oxide was found to be greater compared to bulk in different organs, including the liver ^[24,129]. Similarly, nano magnetite (Fe₃O₄) showed higher bioaccumulation, oxidative stress, and liver tissue damage than its bulk counterpart in another experiment also ^[130]. Orally administered nano maghemite (Fe₂O₃) was found deposited in hepatocytes and kupffer cells, resulting in very little perturbation of biochemical parameters with minimum effects on the liver ^[131]. Histopathological study revealed infiltration of mononuclear cells, ballooning, and hepatic damage with congestion in sinusoids but surprisingly with a decreased level of ALT during the investigation of concurrent effects of aerobic exercise and IONPs in liver enzymes of the treated subject ^[132]. In the rat model administration of coated Fe₃O₄ caused mild liver tissue injury with an altered antioxidant enzyme profile, suggesting oxidative stress-related response ^[133]. Similarly, increased ROS production and decreased cell viability with hampered albumin and urea synthesis in a dose-dependent manner was evident from another study with primary hepatocytes ^[134]. On dose interval treatment with PEG-8000 coated ultra-small superparamagnetic iron oxide nanoparticles (PUSPIOs), have shown temporary alterations in the liver biomarkers and hematological parameters, with lipid peroxidation ^[135]. In a separate experiment, USPIO was found to exhibit more toxic effects on liver tissue than SPIO. In USPIO treated L-02 cells, upregulated expressions of IL-1B, IL-6, IL-18, TNFSF12, TNFRSF12, SAA1, SAA2,

JAK1, STAT5B, and CXCL14 genes with increased secretion of interleukin-6 (IL-6) and altered ER structure due to ER stress supports the occurrence of ER stress-mediated acute-phase inflammatory response that leads to cytotoxicity. Application of ER stress blocker or ATF4 siRNA attenuated the USPIOs effects supporting the involvement of PERK/ATF4 pathway [136]. MAMs (Mitochondria-associated endoplasmic reticulum (ER) membranes), a dynamic microdomain made up of proteins that maintain crosstalk between ER and mitochondria, play a crucial role in Ca^{++} ion and metabolite transfer between two organelles and cellular homeostasis. Both *in vitro* and *in vivo* results suggest SPIO-Nps (iron oxide) accumulation in hepatocytes triggers the overexpression followed by interaction of COX-2 with IP3R-GRP75-VDAC1 complex (inositol 1,4,5 triphosphate receptor, glucose-regulated protein 75, voltage-dependent anion channel 1), the fraction of MAMs that facilitates Ca^{++} transfer. Thereby resulted in profuse Ca^{++} transfer from ER to mitochondria, producing Ca^{++} overload in mitochondria that sparks apoptosis in hepatocytes [137].

Orally administered nano-iron oxide, commonly used in food, disrupts the small intestinal barrier, leading to hepatic lipid metabolism disorders through the gut-liver axis. This disruption causes hepatic damage and iron deposition, impacting lipid homeostasis with decreased phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and increased triglyceride levels. The study highlights the subchronic toxicity of nano-iron oxide and emphasizes the pivotal role of the gut-liver axis in its hepatotoxicity [138]. Fe_2O_3 nanoparticles (E172 food additive) exhibit no evident toxicity in body weight, histopathology, or oxidative stress in animal experiments. However, a sensitive LC-MS/MS-based lipidomic study reveals significant alterations in hepatic glycerophospholipid metabolism, including decreased triacylglycerol and increased phosphatidylcholine. This study enhances understanding of the subacute effects of Fe_2O_3 NPs beyond conventional toxicology assessments [139].

1.3. Graphene Oxide nanoparticles

Because of its special physico-chemical characteristics, graphene oxides are easily produced and tailored to order. They have a wide range of uses in the fields of

electronics, nanomedicine, textiles, water purification, nanocomposite, and catalysis [140-143]. Several investigations unveiled the subacute toxicity caused by GO in different organs including the liver [144,145]. Li *et al* showed that in an SD rat model, GO-induced liver inflammation was associated with lower levels of cholesterol, HDL, and LDL. A separate study with a similar model revealed oxidative stress in accordance with the enhanced ROS production, increased activity of AST/GPT, ALT/GOT, alkaline phosphatase, and LHP (lipid hydro peroxide) with structural alterations in hepatocytes [146]. Varied degrees of histopathological modifications (sinusoidal abnormality, inflammation around portal and central vein, hepatocytic vacuolation) with an elevated level of serum enzyme markers and alterations in MDA, CAT contents concerning oxidative stress indicate GO-induced hepatotoxicity in Wistar rat [147]. GO induced mild early apoptosis and inhibited phase-I drug-metabolism enzymes (CYP3A4, CYP2C9) in upcyte® hepatocytes[148]. Notably, CYP3A4 impairment coincided with an acute-phase response activation. The study highlights the potential health consequences of drug detoxification[148]. Follow table 11 for a comprehensive account.

1.4. Carbon nanotubes nanoparticles

Carbon nanotubes are of two types, single-walled (SWCNTs) with one layer and multi-walled (MWCNTs) with multiple layers. When acid-oxidized MWCNTs (O-MWCNTs) and Tween-80-dispersed MWCNTs (T-MWCNTs) were administered intravenously to mice bodies both types showed inflammatory responses and oxidative stress-mediated liver toxicity. Compared to O-MWCNTs (with carboxyl group), T-MWCNTs (without carboxyl group) exhibited greater effects suggesting hepatotoxicity might be dependent on modification of carboxyl group. Whole genome-wise expression array revealed, upregulated expression of genes related to TNF- α , NF- κ B signaling pathway, NK cell-mediated cytotoxicity, biosynthesis of cholesterol, metabolism by cytochrome P450, GPCRs (G protein-coupled receptors) were recorded for both the treatments[149]. NMR-based metabonomic study unveiled disruption of important metabolic pathways in rat model receiving SWCNTs. Decreased alanine but increased lactate concentration in plasma indicates impairment of amino acid metabolism. Similarly, decreased level of

lipoproteins, and lipids together with the rise in choline, and phosphocholine in serum and liver extract support the disruption of membrane fluidity due to lipid peroxidation.

All these strongly support nanotubes-induced hepatic injury through the modulation of energy, amino acid, and lipid metabolism [150].

Several investigations have revealed that MWCNT resulted in increased ROS production (H_2O_2), and lipid peroxidation (LPO) with a compromised antioxidant defense system (SOD, GPx, GSH, GST), suggesting oxidative stress-mediated hepatotoxicity [4,151,152]. In a series of experiments, Patlolla *et al* had shown that in a dose-dependent manner both carboxylated functionalized carbon nanotubes (SWCNT and MWCNT) exposure to mice resulted in ROS-mediated oxidative stress in association to increased liver biochemical markers and tissue damage [153,154].

Again, MWCNT exposure was found to stimulate pro-inflammatory cytokines (IL-6, IL-1B, COX-1, TNF- α), that serve as an inflammatory mediator to elicit inflammatory responses in the liver [4,151,152]. In an *in vivo* toxicity study, administration of both P-MWCNT (PEGylated) and NP- MWCNT (non-PEGylated) exhibited induction of hepatic inflammation through TNF- α and NF- κ B signaling pathway without any oxidative damage to the liver tissue, though NP- MWCNT shows slightly higher toxicity [155]. Orally administered aqueous extract of *Cinnamomum burmannii* was reported to protect the liver against MWCNT assault by downregulating pro-inflammatory cytokine production and ameliorating the antioxidant system. Suggesting nanotubes triggered liver toxicity is due to oxidative stress and inflammation [4].

Histopathological examinations revealed that MWCNT insult produces clear ultrastructural perturbations including cellular swelling, hydropic degeneration, sinusoidal leukocytosis, sinusoidal space enlargement, vacuolar degeneration, inflammatory cell infiltration associated with focal hepatic and focal perivascular hepatic necrosis, spot necrosis, mitochondrial destruction, congested central vein, macrophage injury even blood coagulation [4,155,156].

Cd-MT (accumulated cadmium-metallothionein) mice when treated with oxidized MWCNTs have shown some striking results. Different doses of MWCNT exposure,

alone promoted the release of free Cd^{++} from Cd-MT, a portion freely available in circulation for elimination while the other portion adsorbed by MWCNT, stayed together in the tissue. Also, co-exposure alleviated hepatotoxicity compared to single exposure [157]. But co-administration of a higher dose of MWCNTs with PbAc in NAFLD (non-alcoholic fatty liver disease) mice resulted in severe liver damage compared to lower combined or single dose (lower or higher) of MWCNTs or PbAc. Remarkable reduction in body weight, liver function, and augmentation of nonalcoholic steatohepatitis (steatosis, lobular inflammation) phenotype was noticed. MWCNTs alone or in combination were found to induce collagen deposition and lipidosis, which leads to hepatic fibrosis. Primary hepatocytes isolated from co-exposed NAFLD mice exhibited a higher rate of apoptosis followed by oxidative stress and inflammation. A significant decrease in expression patterns of p-AMPK α and PPAR γ at combined low doses but reverse expression pattern in the presence of AMPK activators suggests inhibition of AMPK/PPAR γ pathway (adenosine 5'-monophosphate activated protein kinase/peroxisome proliferator-activated receptors γ) may be the reason behind hepatotoxicity [152]. Follow table 12 for a comprehensive account.

1.5. Copper Sulfide/ Cadmium Sulfide nanoparticles

In a study using biomimetic synthesis and ion exchange strategy CuS/CdS nanocomposites were synthesized and tested for hepatotoxicity in liver cells and mice models. *In vitro*, study results unveiled that CuS/CdS nanocomposites cause oxidative stress-mediated apoptosis in liver cells which can be correlated with the perturbed intracellular antioxidant defense system in hepatocytes (SOD & GSH) and excessive accumulation of oxidative products (ROS, GSSG, MDA) that resulted into oxidative stress-mediated apoptosis in both hepatoma cells (BEL7402) and normal liver cells (L-02). Though the first one was more responsive than the latter one. Intravenous injection of nanocomposites to Balb/c mice has shown time-dependent accumulation of Cd^{2+} and Cu^{2+} in the liver, spleen, and kidney. Compared to Cu^{2+} , the liver and kidney retained a significant amount of Cd^{2+} which the physiological system was unable to remove [158]. Compared to CdS microparticles CdNPs exhibited more toxic effects in rat liver.

Greater bioaccumulation of CdNPs leads to the overproduction of metallothionein and ligand formation that has increased its hydrophilicity, facilitating penetration through hepatocyte membrane and such interactions between membrane and NPs further facilitated ROS generation (H_2O_2 , NO) and oxidative stress (lipid peroxidation), disrupting membrane integrity. Biochemical analysis showed increased ALT, AST, and ALP in serum. Ultrastructural study exhibited cytoplasmic degeneration, organellar proliferation (microsome, ER, peroxisome, mitochondria), and extensive parenchymal degeneration suggesting hepatotoxicity^[159].

The hepatic bile salt export pump (BSEP) is crucial for secreting bile salts from hepatocytes to bile and the hepatic MRP2 transporter contributes to bile flow, detoxification, and chemoprotection maintaining a healthy liver. Lowered expression of BSEP mRNA and protein followed by diminished activity of BSEP was observed in the CuSNPs treated group while MRP2 function remain unaltered. Hepatocytes also showed spheroid injury with altered ROS and mitochondrial membrane potential ^[160]. In a separate experiment, different-sized (LNPs - 17.8 nm and SNPs -2.8 nm) copper sulfide nanoparticles ($Cu_{2-x}S$ NPs), biomaterialized with Bovine Serum Albumin were administered in SD rats through tail vein to assess safety and liver toxicity. Both the particles were found to intervene important biochemical pathways including, lipid metabolism, cholesterol/bile acid metabolism, copper ion transport/metabolism, inflammatory and drug metabolism-cytochrome P450 pathway. SNPs are discharged through feces, 7 and 14 days after single administration causing manageable liver toxicity, so it could be a promising nano agent. On the contrary LNPs with more retention power in Kupffer cells, were found to be involved in prolonged and delayed liver toxicity ^[161]. Follow Table 13 for a comprehensive account.

1.6. Cobalt nanoparticle

The human fetal liver cell line L02 demonstrated dose- and time-dependent cytotoxicity following exposure to varying doses of Nano-Co for 12 or 24 h. It has been predicted that cobalt nanoparticles reach hepatocyte intracellular regions through both endocytosis-driven and endocytosis-free pathways. This led to the generation of ROS

and mtROS (mitochondrial reactive oxygen species), which in turn caused oxidative stress damage. Availability of IL-1 β and IL-18 in the extracellular space suggests mtROS-mediated activation of NLRP3 (NOD-like receptor protein 3) inflammasome response, resulting in the upregulation of caspase-1 p20, IL-1 β , and IL-18. Thus Nano-Co induced modulation of ROS/NLRP3 pathway was found to be involved in hepatotoxicity [162]. Follow Table 14 for a comprehensive account.

1.7. Nanoclay particles

In mice, intra-venous administration of nanoclay resulted in acute hepatotoxicity. Elevated level of ALT and AST in serum with routine histological study results indicates toxic effects for higher doses (10 or 20 mg/kg). When co-administered with chemical (carbon tetra chloride, paraquat) or drug (cisplatin) exhibited synergistic increment in liver biomarkers compared to their individual effects [163]. Follow Table 15 for a comprehensive account.

1.8. Nanocellulose (CNS) modified with oxalate ester (NCD)

Structural alteration of nanocellulose (CNS) may increase its application but such modification can lead to toxicity. Short-term exposure of Wistar rat to chemically modified CNS (NCD), mainly higher dose showed an elevated level of ALT, and AST in serum, with increased myeloperoxidase (MPO) but decreased CAT, and GPx activities, indicating disruption in ROS balance. Further over-expressions of iNOS and Bax in treated groups compared to control suggests oxidative stress-mediated inflammation and induction of apoptosis in hepatocytes [29].

1.9. Polystyrene nanoparticles

Polystyrene nanoparticles (PS NP) owe their origin to the degradation of microplastics. In aged -PS NPs (aPS) the oxygen-containing functional groups get increased on its surface. In a recent investigation, comparative toxicity of PS NPs and aPS NPs was done to evaluate their effects on the liver after short-term exposure. Metabolomic, biochemical, and histopathological results reveal that both types of NPs can affect glucose and lipid metabolism through modulating PI3K/AKT/GLUT4 and SREBP-1/PPAR γ /ATGL signaling pathways respectively. Increased glucose but decreased

lipoprotein concentration in serum indicates NPs mediated glycolipid metabolism disruption that provokes the exposed mice to self-regulate various lipoprotein levels in serum. Pyknotic nucleus, congested central vein, unclear sinusoids, vacuolation, hepatocyte ballooning suggests polystyrene NPs mediated liver toxicity [28].

1.10. Chitosan nanoparticles

Chitosan molecules being considered biocompatible have been tested for liver toxicity. Compared to the chitosan molecule, CsNPs showed higher cellular uptake though having poor cell adhesiveness. Availability of more ALT in the extracellular space of BHAL cells after 4 h of exposure indicates loss of membrane integrity. In a concentration-dependent manner CYP3A4 activity was seen to increase suggesting activation of defence mechanism for clearance of CsNPs. Also, it caused significant damage to the nucleus and cytoplasm, indicating necrotic cell death of hepatocytes [27].

1.11. Hydroxyapatite nanoparticles

Hydroxyapatite NPs (HANP) showed antitumor activity in HepG2 cells within a range of 20-80nm particle size. Its cellular uptake and nuclear localization followed by efficacy was found to diminish with increasing particle size. Treated cells exhibited caspase-3, and caspase-9 activation with increased proapoptotic markers (Bax, Bid) and with a concomitant decrease in Bcl-2 and cytochrome c release from mitochondria to the cytoplasm, confirmed HAPN-mediated activation of mitochondrial-dependent apoptotic pathway [164]. A similar result was documented in another *in vitro* experiment, where incubation of buffalo rat liver (BRL) cells with 80 nm HANPs at 200 µg/mL, exhibited diminished cell viability, LDH leakage, induced apoptosis, and necrosis, and MAPK pathway-mediated cytotoxicity. *In vivo*, study results showed infiltration of inflammatory cells near the portal area, increased WBC count, ALT, AST, and TNF-α in serum of treated rats with increased levels of H₂O₂, MDA suggesting HANPs induced oxidative stress-related liver injury [165]. Follow Table 16 for a comprehensive account.

1.12. Quantum dots

Mice with both acute and chronic exposure to cadmium selenium (CdSe) QDs showed predominant liver accumulation. Enlarged central vein and disordered hepatic cords

were observed for chronic exposure only. In contrast the *in vitro* study unveiled that, Hepa 1-6 cells (murine liver cells) became condensed and decreased in size while J774A.1 cell (macrophage -substitute for Kupffer cell) became condensed and round. Beta-mercaptoethanol (β -ME) pretreatment was found to attenuate the QDs-induced increase of MDA level, suggesting QDs-induced oxidative stress in the liver involves the production of free radicals with compromised ROS scavengers (GSH-Px) that have provoked cytotoxicity in hepatocytes and macrophages, potentiating impairment of cellular differentiation without causing any death [166]. Similarly, perturbed redox homeostasis in mice treated with Cd/Se/Te-based quantum dot 705 has been documented. Increased levels of copper, zinc, and selenium with trace elements and their corresponding transporters (ZIP8, ZIP14, and CTR-1), over-expressed oxidative stress markers (heme oxygenase-1 expression, 8-oxo-7,8-dihydro-2'-deoxyguanosine) along with reduced SOD, GPx activity, GSH/GSSG ratio indicates oxidative stress. Also upregulated pro-inflammatory mediators (IL-6, TNF- α) and liver markers (ALT, AST) signify liver damage due to oxidative stress-mediated inflammatory response [167]. CdSe/ZnS QDs were also reported to induce oxidative stress, inflammation, pyroptosis, and liver dysfunction. Application of Z-YVAD-FMK (caspase-1inhibitor), 2-APB (Ca²⁺ channel blocker), BAPTA-AM (intracellular Ca²⁺ chelator), NAC (a total ROS scavenger), Mito-TEMPO (a mtROS scavenger) and further silencing NLRP3 was reported to alleviate QDs mediated pyroptosis of hepatocytes, confirming the underlying mechanisms includes intracellular Ca²⁺ mobilization that triggered mtROS generation and subsequent activation of NLRP3 inflammosome leading to caspase-1mediated pyroptosis. A similar result was in agreement when NLRP3 knocked out mice exposed to QDs [168]. On the contrary except QDs accumulation in mitochondria, lysosome, and lipid droplets no significant signs of liver damage were observed when Kunming mice were subjected to Mn-doped ZnS QDs and polyethylene glycol-coated QDs exposure[169]. Similarly except slight increment of liver markers (ALT, AST, ALP) in serum, no such remarkable liver tissue damage was recorded in mice exposed to cadmium-free indium-based QDs [25]. Again, cadmium telluride (CdTe) QDs

administration was found to elevate oxidative stress in AML 12 (murine hepatoma cells alpha mouse liver 12) and mice model, concomitant increased expression pattern of the tumor-suppressor gene (p53), proapoptotic gene (Bax) and decreased level of antiapoptotic marker (Bcl-2) suggests activation of mitochondria-mediated apoptotic pathway in hepatocytes. NF-E2-related factor 2 (Nrf2) deficiency was found to attenuate CdTe-QDs provoked injury and apoptosis suggesting the underlying mechanism involves modulation of the Nrf2 signaling pathway [170]. A series of investigations have proved that mitochondria are the prominent target of CdTe-QDs in hepatocytes. In different cell lines and mice models, it was found that interaction between CdTe-QDs and mitochondrial membrane resulted in mitochondrial enlargement, membrane potential disruption, opening of permeability transition pore (PTP), impaired oxidative phosphorylation *via* diminishing activity of ETC enzymes, ROS accumulation, redox damage, ATP depletion and increased PGC-1 α . Together all these indicate oxidative mediated stress-mediated release of cytochrome c and Bax to promote intrinsic and extrinsic pathways of apoptosis in CdTe-QDs exposed hepatocytes [9,171–173]. When normal and carcinoma liver cells were incubated with CdTe/CdS QDs for 24hrs., both the cells showed similar lysosomal accumulation of QDs followed by abnormal activation of lysosomal enzymes that triggered lysosome-dependent ROS production and autophagy. Inhibition of lysosomal enzymes were also found to prevent ROS production and activation of autophagic flux and thereby rescued hepatocytes from cytotoxic effects of QDs [3]. A recent *in vivo* investigation unveils the sub-acute low dose of CdTe QDs uptake leads to both activation of NF-KB pathway through overproduction of ROS that also indirectly regulates NLRP3 inflammasome assembly to trigger inflammatory cascades *via* inflammatory cytokines (IL-1 β , TNF- α , IL-6) and activation of Kupffer cells to cause liver tissue injury. In *in vitro* study pretreatment of KUP5 cells with NAC (N-acetylcysteine – ROS scavenger) and DHMEQ (Dehydroxymethylepoxyquinomicin- NF-KB translocation inhibitor) before QDs, reversed the activation of Kupffer cells following down-regulation of NF- κ B, caspase-1, and NLRP3 [174]. A recent study highlights the varied impact of CDs (Carbon Quantum

Dots) on liver cells (KUP5 and AML12 cells *in vitro*) and the importance of the TFEB-lysosome pathway in regulating autophagy and apoptosis induced by CDs on liver cells for a comprehensive toxicological safety evaluation [175].

1.13. Gold nanoparticles

Gold is generally unreactive in its natural state but becomes reactive in its ionic form. It can also exist as gold salts, allowing the synthesis of nanomaterials with properties like easy synthesis, high particle reactivity, and strong optical characteristics. [176,177]. In recent days, gold nanoparticles (AuNPs) have gained considerable attention in various fields, especially in biomedical sciences due to their unique physicochemical properties [178]. Nevertheless, there are many concerns regarding their potential hepatotoxic effects that have raised questions about their safety use in such applications. Numerous inflammatory and cytotoxic responses have been observed with smaller-sized AuNPs in comparison to contact with larger-sized AuNPs with the same mass concentration because of their highly reactive role with biological constituents, and have stressed the harmful effects produced by a large number of nanoparticles [179]. AuNPs activate hepatic macrophages and consequently stimulate the occurrence of immune hepatitis and liver dysfunction [180,181]. Serum ALT and AST levels, indicative of liver damage, remained within the normal range in NC (Normal Chow) diet-fed mice 24h or 7d after AuNP administration, suggesting AuNPs' non-toxicity under normal diet conditions [182]. Conversely, MCD (methionine and choline-deficient) diet-fed mice exhibited elevated ALT and AST levels post-AuNP administration, indicating hepatotoxicity. The experiment revealed that MCD diets induced hepatic TG accumulation through the inhibition of mitochondrial beta-oxidation and blocking hepatic export of very low-density lipoprotein, but AuNP-induced hepatotoxicity was attributed to increased inflammatory response and apoptosis, not accumulated TG contents [182]. Intravenously injected AuNPs rapidly accumulate in Kupffer cells in the liver, stimulating these cells and leading to increased monocyte function, upregulated cytokine secretion, and subsequent liver damage through enhanced necrosis, apoptosis, and abnormal ROS production [183]. The toxicity of AuNPs is associated with their capacity to stimulate

inflammatory responses and accelerate stress-induced apoptosis, with smaller nanoparticle sizes contributing to toxicity [184]. AuNPs induce hepatocellular injury through ROS generation, promoting oxidative stress [185]. This oxidative stress, characterized by lipid peroxidation, protein damage, and DNA modifications, is exacerbated by inflammatory responses and pro-inflammatory cytokines. The correlation between nanoparticles and oxidative stress suggests fatty acid peroxidation as a probable cause for AuNP-triggered DNA destruction [186]. Haseeb *et al* measured oxidative stress markers in rats exposed to AuNPs, revealing increased MDA levels specifically in the liver, indicating AuNPs' liver-specific oxidative stress [187]. The mutagenic and carcinogenic nature of MDA, a product of fatty acid peroxidation, suggests its potential to combine with DNA, leading to DNA damage and potentially activating programmed cell death pathways [187]. Research has shown that AuNPs can enter hepatocytes through various mechanisms, including endocytosis and direct penetration of the cell membrane [188,189]. Once internalized, these may accumulate in specific subcellular compartments, such as the endoplasmic reticulum (ER) or mitochondria which leads to inducing organelle-specific toxicity. The disruption of cellular organelles can trigger a cascade of events leading to hepatocellular damage [190,191]. Cell migration, crucial for mammalian cell survival and differentiation and regulated by external signals, was significantly reduced by 70% in HeLa cells treated with MUAM-AuNPs, as demonstrated in a gap-filling assay by Lee *et al* (2019); this reduction was attributed to the loss of long F-actins aligned with the migration axis, impacting migration-related signaling pathways, disrupting extracellular matrix organization, and ultimately impeding cell migration [192–194]. Additionally, AuNPs induced differential gene expression in treated samples, involving both upregulated and downregulated genes associated with cellular metabolism, protein catabolism, cell cycle, and G1/S transition; notably, downregulation of genes related to the G1 phase and nucleic acid metabolism suggested inhibition of DNA synthesis. In a separate experiment, 1.4-nm triphenyl monosulfonate (TPPMS)-coated AuNPs caused necrotic cell death through elevated oxidative stress and loss of mitochondrial potential, while

Tiopronin-coated AuNPs induced necrosis *via* increased ROS production and apoptosis due to mitochondrial dysfunction; citrate AuNPs also exhibited dose-dependent ROS production leading to apoptosis [195,196]. Moreover, Au clusters significantly increased ROS production by inhibiting TrxR1 activity, inducing apoptosis, and disrupting mitochondrial membrane polarization [197]. Finally, irradiation in the presence of AuNPs led to an interaction with the cell membrane protein disulfide isomerase (PDI), disrupting thiol balance, causing cellular redox imbalance, and ultimately inducing oxidative stress. [196]

1.14. Silver nanoparticles

Silver nanoparticles (AgNP)-intoxication significantly disturbs normal liver function, elevates hepatic lipid peroxidation, increases liver DNA damage, and induces biochemical and histological alterations in rats [198]. The toxicity of AgNPs mainly originates from the degraded forms of AgNPs, the “particle-specific effect” or the triggered oxidation stress [199]. After cellular intake, these (AgNPs) would enter the acidic endo/Lysosomes (pH4.5–6.5) and undertake chemical transformation from particulate silver to elemental silver, Ag⁺, Ag-O- and Ag-S- species [200]. The Ag⁺ released from AgNPs dissolution is thought to bind intracellular sulfhydryl group (–SH)-containing molecules and leads to cytotoxicity, which is known as the “Trojan-horse” mechanism [199]. AgNPs also help in intracellular reactive oxygen species (ROS) production and cause cellular damage, *e.g.* genotoxicity, mitochondrial dysfunction, and cell membrane damage [201]. Ag ions have been reported to cause disturbance and destruction of mitochondrial function through interaction with thiol groups of inner mitochondrial membrane proteins and AgNPs decrease the activity of mitochondrial respiratory chain complexes and reduce antioxidant factors like glutathione, thioredoxin, superoxide dismutase, and N-acetylcysteine in liver cells [202,203]. Xu *et al* (2019) investigated two normal hepatic cell lines (NCTC1469 and L-02) and two hepatoma cell lines (Hepa1-6 and HepG2) to assess the cytotoxicity of AgNPs. They have shown AgNPs could certainly lead to intra-cellular oxidation stress and cytotoxicity through acting Glutathione S-transferases (GST) molecules and thus

suppressing its enzyme activity, although GST expressions were not significantly affected^[201]. The research also highlighted the binding of High Molecular Weight proteins to Ag⁺ became saturated and more Low molecular weight molecules (e.g. metallothionein) were continually synthesized by cells to neutralize AgNPs and Ag⁺ for detoxification. It indicates that the dissolution of internalized AgNPs resulted in the formation of Ag-protein complexes. As a consequence, the damage of protein molecules by AgNPs and Ag⁺ would destroy the intra-cellular homeostasis of the liver. Assar *et al* (2022) pointed out that after 15 and 30 days of exposure to the maximum dose of AgNPs in rats, a drop in liver weight was observed to a striking rise in lipid peroxidation, leading to structural changes to lipid vacuoles^[204]. This finding also showed that a state of oxidative injury was provoked by silver nanoparticles in a dose-dependent way by the raised hepatic MDA (malondialdehyde) levels and the depletion of the antioxidant defensive mechanism by reducing the hepatic reduced glutathione (GSH) levels. The most severe hepatic damage was associated with increasing the AgNP-administered dose and expanding exposure time. Research findings from Piao *et al.* (2011), Li *et al.* (2017), Ansar *et al.* (2017), and Li *et al.* (2020) have detailed that continuous elevation of Ag⁺ concentration leads to continuous induction of hydroxyl radical, ultimately consumes more intracellular GSH, and disturbing the homeostasis of free radical scavenging^[205–208]. AgNPs raised MDA levels causing oxidative damage in rats^[209]. Many studies support that the liver is the main target organ for AgNP action. The histological assessment of the liver indicated pathological changes that were dose and time-dependent and happened in the liver after 30 days of increasing concentrations of AgNP exposure. Elje *et al* (2020) showed that low levels of dissolved Ag were found in the Ag-NPs exposure dispersions shortly after exposure in the HepG2 human liver cells, and the amounts were lower than the measured EC50 for cytotoxicity of AgNO₃^[111]. An increased intracellular level of ROS can activate cell-death-regulating pathways, such as p53, AKT, and MAP kinase^[185]. Several studies identified six genes from HepG2 Liver cells, with three showing significant up-regulation of FOS and JUN, and two demonstrating up-regulation of EGR1, CXCL8, HSPB1, and MT2A. Notably,

high-dosage AgNP exposure increased fold changes in genes associated with cell proliferation (FOS, JUN, and EGR1) [210]. Microscopic images revealed nuclear membrane distortion, blebbed nuclei formation, and accumulation of autophagic vacuoles in AgNP-treated liver cells, along with increased mitochondria, cytoplasmic vacuoles containing silver nanoparticles, and swollen lipid droplets. In hepatocytes, CEBPA (CCAAT enhancer binding protein alpha) is highly expressed and plays a critical role in regulating many metabolic liver genes, while CEBPB (CCAAT enhancer binding protein beta) is up-regulated during liver regeneration and plays a crucial role in the development of liver or acute inflammatory response [211]. The proto-oncogenes FOS and JUN, which are known to play important roles in both cell survival and the signaling pathway involved in hepatotoxicity, were highly up-regulated in the presence of AgNPs. In addition to that, heat shock protein family members HSPB1, HSPA4L, and HSPH1 were also significantly up-regulated [212]. Xin *et al*, (2015) reported that AgNPs induced oxidative stress, and consequently increased expression of heat shock protein and heme oxygenase (HMOX1) in both liver and lung cells [213]. Sooklert *et al* (2019) identified 24 interesting candidate genes as possible targets of AgNP-induced hepatocellular toxicity. SOX15, a highly upregulated gene, acts as a transcription activator involved in embryonic development regulation and cell fate determination [210]. TLL1, the most noticeable down-regulated gene, is necessary for various developmental events. AgNPs may exert cytotoxic effects through SOX15 upregulation or TLL1 downregulation in hepatic cells. Deregulated autophagy after AgNP treatment was also seen which may lead to increased cell death either independently or synergistically with apoptosis or necrosis [214]. Wen *et al* (2022) and Recordati *et al* (2016) observed increased hepatocellular necrosis and gall bladder hemorrhage in mice injected with AgNPs, particularly with 10nm AgNPs. AgNP administration induced exacerbated hepatic steatosis, heightened liver injury, and elevated risk of NAFLD development and progression [215,216]. The effects were attributed to hyperactivation of SREBP-1c-mediated de novo lipogenesis, pro-inflammatory cytokine activation, and increased oxidative stress and DNA methylation [216]. Kim *et al* demonstrated that cAgNPs (citrate-coated

and stabilized) caused significant changes in ALP and LDH levels, indicating liver tissue damage persisting up to 28 days after exposure and suggesting prolonged impairment of liver structure and functions following a single exposure^[217]. From Lee *et al* (2013), it was reported that the deposited AgNPs in hepatocytes were found to be individual particles with a size smaller than 100 nm in diameter^[218]. AgNPs accumulated in hepatocytes' endosomes and lysosomes, with additional deposition in Kupffer cells (>100 nm agglomerates)^[218]. Kupffer cells played a role in inflammation observed with mild inflammatory cell infiltration in portal vein areas. Elevated ALT and AST levels indicated liver damage persisting up to one month after AgNP administration^[218]. Maternal exposure to AgNPs *via* the intragastric route led to increased silver content in rat offspring livers, causing a significant reduction in body weight and dilated blood vessels. Liver damage, indicated by vacuolation and lipid peroxidation, was associated with elevated caspase-9 concentration, suggesting AgNPs induce apoptosis through the intrinsic pathway in offspring livers^[219].

CONCLUSION

In summary, the extensive examination sheds light on the intricate landscape of hepatotoxicity induced by various nanoparticles (NPs), revealing distinct mechanisms and effects associated with different nanomaterials. Size-dependent hepatotoxicity is observed in SiNPs, with smaller particles causing more severe liver injury. The combined toxicity of SiNPs with other liver toxins highlights potential synergies in NP-induced liver damage. Oxidative stress, inflammation, apoptosis, and genotoxicity are induced by NiO-NPs, WO₃ NPs, Nano-CuO, and other nanomaterials, illustrating the complexity of NP-mediated hepatotoxic effects (refer to Fig 1, 2, and 3).

Integrative omics analyses identify key proteins and disrupted metabolic pathways in SiNP-induced hepatotoxicity, underscoring the necessity for a multifaceted understanding of NP-induced liver damage. Carbon nanotubes (CNTs), including SWCNTs and MWCNTs varieties, contribute to hepatotoxicity through inflammatory

responses and oxidative stress, with variations in toxicity observed among different types of CNTs.

Moreover, exposure to CuS/CdS-NPs, cobalt nanoparticles, nanoclay particles, nanocellulose, polystyrene nanoparticles, chitosan nanoparticles, hydroxyapatite nanoparticles, quantum dots, and gold nanoparticles elucidates diverse hepatotoxic effects, underscoring the importance of considering nanoparticle characteristics in toxicity assessments.

Despite these toxicities, it is noteworthy that nanoparticles play a pivotal role in diverse biomedical applications, showcasing their versatility and impact. In cancer therapy, catalytic strategies employing substances like hydrogen peroxide and glucose, alongside biocompatible nanomaterials, promise efficient treatment with minimal side effects^[220]. Nanomaterials contribute significantly to the fight against COVID-19, aiding in rapid diagnostics, vaccine development, and therapeutic interventions^[221]. Transition metal-based nanoparticles, particularly those with anisotropic shapes, offer unique properties for biomedical applications, including drug delivery and imaging^[222]. Precision nanoparticles (PNPs) emerge as discrete structures with precisely tailored heterogeneity, addressing challenges associated with uncontrolled nanoparticle variability^[223]. PNPs significantly enhance the performance of nanoparticle-based vehicles in various biological processes, presenting a promising avenue for improved biomedical outcomes.

Therefore, the study concludes by emphasizing the urgent need for a comprehensive understanding of NP-induced hepatotoxicity to ensure the safe use of nanomaterials, suggesting further *in vivo* studies and exploration of potential protective strategies. Additionally, the proposal of herbal gold nanoparticles as a potential hepatoprotective agent opens avenues for future research and development in the field. Overall, the findings underscore the complexity and diversity of nanomaterial-induced hepatotoxicity, emphasizing the importance of continued research for safer nanomaterial applications in various contexts.

Abbreviations

OH- hydroxyl radical, Akt-serine/threonine kinase, ALB - albumin, ALP- alkaline phosphatase, ALR- autophagic lysosomal reformation, ALT- alanine aminotransferase, AMPK-AMP activated protein kinase, aPS- UV aging Polystyrene, AST- aspartate aminotransferase, Atf6-activating transcription factor 6, ATGL- adipose triglyceride lipase, ATP- adenosine triphosphate, Bag4-BAG cochaperone 4, Bax- Bcl-2 associated X protein, Bcl2- B-cell lymphoma 2, Bid-BH3 interacting-domain death agonist, BUN- Blood urea nitrogen, CAT- catalase, CHOP- C/EBP Homologous Protein, COX-2 - cyclooxygenase-2, CRP-C reactive protein, Cyp2B19-Cytochrome P4502B19, Cyp2C50- Cytochrome P4502C50, CYP3A4- Cytochrome P4503A4, DNA-deoxy ribonucleic acid, ECM-extra cellular matrix, EIF2AK3 - eukaryotic translation initiation factor 2-alpha kinase 3, eIF2 α -eukaryotic initiation factor 2 α , EMT-epithelial mesenchymal transition, ER-endoplasmic reticulum, ETC- electron transport chain, Gab1-GRB2 associated binding protein 1, GADD153-growth arrest and DNA damage 153, GGT- gamma glutamyl transferase, GGT- gamma-glutamyl transpeptidase, GLUT4-Glucose transporter 4, GPx - glutathione peroxidase, GR- glutathione reductase, grp -glucose regulated protein 78,94, GRP75 - glucose regulated protein 75, GRP78- glucose regulated protein 78, GSH-glutathione, GSH Px - glutathione peroxidase, GSSG - glutathione disulfide, GST- glutathione S-transferase, Gsta2- glutathione S-transferase, α 2, H₂O₂-hydrogen peroxide, HD - high dose, HDL-C- high-density lipoprotein, HIF-1 α -hypoxia inducible factor 1, HO 1/ Hmox I -heme oxygenase 1, HSP-70-heat shock protein 70, IgG-immunoglobulin G, IKK1,2- I κ B kinase, IKK- α - I κ B kinase α , IL-1 α - interleukin 1 α , IL-1 β - interleukin 1 β , IL-4,6- interleukin-4,6, iNOS- inducible nitric oxide synthase, IP3R- inositol 1,4,5 triphosphate receptor, Ire1 α - inositol-requiring enzyme type 1 α , I κ B(α) -inhibitor kappa B α , JNK-jun N-terminal kinase, LD - low dose, LDH - lactate dehydrogenase, LDL-C- low-density lipoprotein, LHP-lipid hydroperoxide, LPO-lipid peroxidation, MAPK- mitogen activated protein kinase, MDA- malondialdehyde, mdm²-murine double minute 2, Mfn1-mitofisin-1 miR210-miRNA210, MMP9-matrix metalloproteinase 9, MMP-

mitochondrial membrane potential, MnSOD-manganese superoxide dismutase MPO-
myeloperoxidase, MT 1 – metallothionein 1, mTOR - mammalian target of rapamycin,
NADPH-reduced nicotinamide adenine dinucleotide phosphate NAFLD- non-alcoholic
fatty liver disease, Ncf-1,2-neutrophil cytosolic factor 1,2, NF-κB- nuclear factor kappa
beta NIK- NF-κB-inducible kinase, NLRP3 - NOD-like receptor protein 3 NO-nitric
oxide, Nrf2- nuclear factor erythroid 2-related factor 2 O-MWCNT-acid-oxidized multi-
walled CNTs, Opa 1-optic atrophy 1 p21-cyclin-dependent kinase inhibitor 1, p38-
protein kinase, p53-tumor suppressor protein p53, p-AKT- phosphoprotein kinase B,
pdi-3 - protein disulfide isomerase -3, PERK- protein kinase RNA like ER kinase,
PERKIN-E3 ubiquitin-protein ligase PGC1α- peroxisome proliferator-activated receptor
gamma coactivator 1 alpha PI3K-phosphatidylinositol 3-kinase, PINK- PTEN induced
kinase PPARγ- peroxisome proliferator-activated receptor-γ, PS- Polystyrene, ROS -
Reactive oxygen species, Smad2-suppressor of mothers against decapentaplegic2, SOD-
superoxide dismutase, SREBP-1-sterol regulatory element binding protein-1, STAT-3 -
Signal transducer and activator of transcription 3, T AOC - total antioxidative capacity,
TBA - total bile acid, T-Bil-total bilirubin, TC- total cholesterol, TFAM- mitochondrial
transcription factor A, TG - triglyceride, TGF-β1-transforming growth factor 1 beta,
TIMP1-TIMP metalloproteinase inhibitor 1, TLR2,4- toll like receptor 2,4 T-MWCNT-
Tween-80-dispersed multi-walled CNTs, TNF-α- tumor necrosis factor alpha, TNOS-
total nitric oxide synthase, TP-total protein, TSOD - total superoxide dismutase, TT-total
thiol, VDAC1 - voltage-dependent anion channel 1, xbp-1 - X box binding protein-1,
XBP-1S- X box binding protein-1S, α-SMA-alpha smooth muscle actin, γGT - gamma
glutamyl transferase.

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