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Toxicity of Nanoparticles on The Spleen in Animal Studies: A Scoping Review

(Studi Toksisitas Nanopartikel Organ Limpa pada Hewan Percobaan, Tinjauan *Scoping Review*)

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Abstract: Nanotechnology has been developing in the medical field, but some nanoparticles have toxic effects on the body, including the spleen. This scoping review represents an attempt to take stock of existing research results related to the presence or absence of toxicity to the spleen caused by nanoparticles involving experimental animals. A scoping review was conducted to synthesize and map the toxicity of nanoparticles. It has been searched on PubMed databases for spleen or lien and toxic or toxicity, and nanoparticles or dendrimers or "metal nanoparticles" or “magnetite nanoparticles” or nanoshells or “multifunctional nanoparticles” or nanocapsules or nanoconjugates or nanodiamonds or nanogels or nanospheres. Seventeen studies met our inclusion criteria. In conclusion, it showed that 13 nanoparticles could cause toxicity in rodent spleen and as many as 4 nanoparticles did not cause toxicity in rodent spleen.

Keywords: Nanoparticles, rat, spleen, toxicity

Abstrak: Nanoteknologi telah berkembang di bidang medis, namun beberapa nanopartikel memiliki efek toksik pada tubuh seperti limpa. Tinjauan *scoping review* ini merupakan upaya untuk mengambil hasil-hasil penelitian yang sudah ada terkait dengan ada tidaknya toksisitas pada limpa yang disebabkan oleh partikel nano yang melibatkan hewan percobaan. Tinjauan *scoping review* dilakukan untuk mensintesis dan memetakan toksisitas nanopartikel. Hasil penelitian di cari melalui PubMed dengan kata kunci: *spleen* OR *lien* AND *toxic* OR *toxicity* AND *nanoparticles* OR *dendrimers* OR *metal nanoparticles* OR *magnetite nanoparticles* OR *nanoshells* OR *multifunctional nanoparticles* OR *nanocapsules* OR *nanoconjugates* OR *nanodiamonds* OR *nanogels* OR *nanospheres*. Tujuh belas studi memenuhi kriteria inklusi. Kesimpulannya, menunjukkan bahwa 13 nanopartikel dapat menyebabkan toksisitas pada limpa hewan pengerat dan sebanyak 4 nanopartikel tidak menyebabkan toksisitas pada limpa hewan pengerat.

Kata kunci: Limpa, nanopartikel, tikus, toksisitas

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INTRODUCTION

THE application of nanotechnology in the medical field or nanomedicine to date has become more extensive(1). Nanomedicine is in high demand in the field of healthcare because nanoparticles can function as a nanocarrier to protect drugs from degradation and possibility of uncontrolled drug release. Nanoparticles can deliver drugs to specific target cells, reduce drug clearance, and improve drug accumulation in targeted tissue, resulting in increased therapeutic effects and reduced side effects. In addition, the tiny size of nanoparticles results in high solubility and bioavailability that enable them to easily cross the BBB (Blood-Brain Barrier) and enter the pulmonary system as well as be easily absorbed through the tight junctions in endothelial cells. Nanoparticles can also function as an antimicrobial. They have a surface area that fits perfectly into the surface of microbial cells, and they can damage the cell walls and DNA of many microbes(2). However, nanoparticles are potentially toxic and harmful to living organisms although this depends on the type, size, concentration, solubility, stability, as well as chemical and physical properties of each type of nanoparticles(3).

Nanoparticles in the blood are normally filtered by Kupffer cells of the liver, macrophages in the spleen, and the kidney. Once entering the body, nanoparticles are recognized as a foreign substance. The immune system, one of which parts is the macrophages, will consequently work to degrade this foreign substance, thereby involving the spleen as one of the organs that play a role in the pharmacokinetics of nanoparticles(4). The spleen also acts as a filter for the blood and coordinates immune responses. In such coordination, the spleen has the white pulp and red pulp that benefit the removal of damaged or aged blood cells. As one of the main organs in the immune system, the spleen becomes a target organ for nanoparticle toxicity. Some evidence suggests that a large majority of nanoparticles can effortlessly interact with the immune system, particularly those entering the spleen. This interaction potentially has immunotoxic effects on the spleen, thereby resulting in disease susceptibility. A study by Zhou *et al.*(5) proved that the immunotoxicity in the spleen is marked by decreases in T cells (CD3+), Th cells (CD3+, CD4+), and cytotoxic T cells, which are thought to be caused by a signalling pathway through the activation of MAPK. Such activation can increase the production of proinflammatory cytokines and ROS (Reactive Oxygen Species) which then damage the spleen cells(5).

Previous literature mentioned one type of nanoparticles, i.e. copper nanoparticles (CuONPs), that can cause toxicity to the spleen due to their excessive accumulation. Accumulated CuONPs interact with both intracellular and extracellular molecules through the signalling pathway. Such interaction can easily lead to an increase in proinflammatory cytokines and a decrease in the weight of the spleen. This weight loss indicates the presence of toxicity in the spleen(6). It is important to know the toxicity associated with the spleen because the chemical compounds that cause poisoning of erythrocytes so that they are destroyed in the spleen can also cause damage to the spleen and the development of tumors(7). In addition, drugs that can cause enlargement and blockage of blood vessels in the spleen, can also cause damage to other organs such as the liver(8). The researchers have not found hitherto any literature that summarizes which nanoparticles can damage the spleen, particularly in experimental animals. Therefore, this review aims to map the results of existing research on the presence or absence of toxicity to the spleen of experimental animals caused by nanoparticles.

MATERIALS AND METHODS

MATERIALS. The material used in this study was the article or study of toxicity to experimental animals' spleen caused by nanoparticles published before 27 May 2020. The articles were obtained from Pubmed.

METHODS. This study used a scoping search method. In scoping review, the authors do not assess the quality of studies reviewed. In the first step, we identified the keyword of research using MeSH in MEDLINE to determine the terms. The references obtained from authentic articles accessed through a credible source on the website https://pubmed.ncbi.nlm.nih.gov/. The search for academic journals was conducted on 27 May 2020 in NCBI using the keywords (((spleen [Title/Abstract]) OR (lien [Title/Abstract]) AND ((toxic[Title/Abstract]) OR(toxicity[Title/Abstract]))) AND ((((((((((nanoparticles[Title/Abstract]) OR (Dendrimers [Title/Abstract])) OR (Metal Nanoparticles [Title/Abstract])) OR (Magnetite Nanoparticles [Title/Abstract])) OR (Nanoshells [Title/Abstract])) OR (Multifunctional Nanoparticles [Title/Abstract]))OR(Nanocapsules Title/Abstract])) OR (Nanoconjugates [Title/Abstract])) OR (Nanodiamonds [Title/Abstract])) OR (Nanogels [Title/Abstract])) OR(Nanospheres [Title/Abstract]).

The inclusion criteria comprised articles reporting the studies that used animal subjects with administration of particular nanoparticles and a comparison with the control, examined the presence or absence of damage of or accumulation in the spleen, or examined the spleen weight and oxidative stress parameters. This literature review did not include studies in humans due to the limited number of such articles and the difficulty in examining the morphology of the human spleen. The spleen toxicity in this study refers to the significant changes in 1) the accumulation of nanoparticles in the cells or interstitial cells of the spleen and or 2) changes in the weight of the spleen or 3) the accumulation of nanoparticles in the spleen completed with histological features that indicate damaged spleen, such as inflammation, decreased lymphocyte count, necrosis, or decreased pulp size. Meanwhile, the exclusion criteria consisted of articles that could not be accessed in full text, used a non-English language, and involved an additional modification of nanoparticles, such as addition of a protective capsule or nanoparticle wrapping, thereby causing the loss of the natural properties of nanoparticles.

RESULTS AND DISSCUSSION

The search using the predetermined keywords resulted in 117 related journals. Following the determination of the inclusion criteria, a total of 20 journal articles were found to fulfil the criteria with 3 articles being excluded, leaving 17 journal articles to be discussed in this scoping review (Figure 1). Based on such operational definition, 13 nanoparticles were found to cause toxicity while 4 other nanoparticles induced no toxicity to the spleen (Table 1).

Entry Mechanism of Nanoparticles into the Spleen. The majority of nanoparticles are not excreted into the urine, but instead they are eliminated into the RES (reticuloendothelial system), such as the liver and spleen[9]. Furthermore, the splenic circulation is highly permeable, thus allowing some molecules to easily enter the tissue. The spleen is also an organ with the largest amount of blood supply, reaching approximately 170 mL/min/100 g, and more than 90% of the splenic blood will flow into the white pulp bypassing the red pulp. Therefore, a higher amount of blood supply to the spleen leads to greater accumulation of nanoparticles[2].

The splenic circulation consists of two different parts, each supplying blood to the white pulp and the red pulp. The white pulp is surrounded by a zone named the marginal zone (MZ) that contains numerous parts of the immune system, including IgM+/IgD, B lymphocytes, macrophages, and lymphocytes. When blood flows into the spleen, its speed declines and the nanoparticles contained therein will easily interact with the immune system, indicating that the spleen affects the pharmacokinetics of nanoparticles. The interaction of nanoparticles with the immune system in the spleen can excessively activate proinflammatory cytokines and lead to other undesirable toxic effects. Such effects will cause damage to the cells and tissue of the spleen. However, nanoparticles in the spleen can be either toxic or non-toxic depending on the size, concentration, dose, properties, and amount of nanoparticle accumulation in the spleen tissue[4].

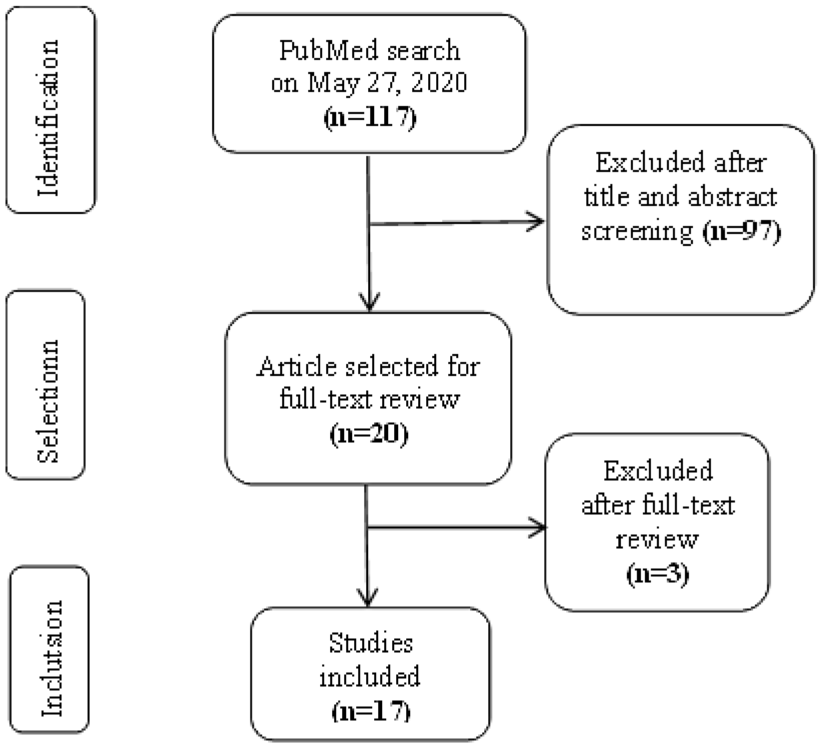

Figure 1 Flow chart for study selection.

Table 1. Findings of the studies that fulfilled the inclusion criteria.

Author	Nanoparticles	Dose	Duration of Administration	Spleen Tests	Results of Spleen Tests
Lopez-Chaves *et al.,* 2018	AuNPS	Water/AuNPs size 10 nm/30 nm/60 nm in 50 mg/L water (IP)	Unknown	ICPMS of spleen (+) Spleen weight (-) Histopathology (-)	Accumulation of AuNPs in cytosol and cell nucleus (S)
Xia *et al.,* 2019	AuNPs	PBS/AuNPs size 5 nm/20 nm/50 nm (IV)	5 times (5 days)	ICPMS of spleen (+) Spleen weight (-) Histopathology (-)	Intracellular accumulation of AuNPs (S)
Dey *et al.*, 2019	S1NP* S2NP* CuONPs	PBS/S1NP & S2NP PBS concentration of 100/200/500/1000 ug/kgBW (IP)	3 times a day (15 days)	AAS of spleen *(+)* Spleen weight (+) Histopathology (-)	Accumulation of Cu in tissue (S) Spleen weight << (S)
Feng *et al.,* 2018	IONPs	PBS/IONPs concentration of 1.5/2.5/5 mg Fe/kgBW (IV)	Once	ICPMS of spleen(+) Spleen weight (-) Histopathology (+)	Accumulation of iron in tissue (S) Plasmacytosis (NS)
Pham BTT *et al.,* 2018	s-SPIONs	PBS/SPIONs size 10 nm/25 nm/concentration of 90 mg Fe/kgBW (IP)	7 days	ICPMS of spleen(+) Spleen weight (-) Histopathology (+)	Accumulation SPIONs (S) Histopathology (NS)
Kalateh *et al.*, 2019	ZnO-NPs*	Free ZnO/ZnO concentration of 100/ 200/300 mg kg^{-1} (oral)	Daily (28 days)	AAS of spleen (+) Spleen weight (+) Histopathology (-)	Accumulation of zinc in tissue (S) Spleen weight >>(S)
Savery *et al.*, 2017	AgNP*	PBS/AgNP concentration of 0.0082/0.0025/0.074/ 0.22/0.67/2.0/6.0 mg kg^{-1}, size 20/100 nm AgNP in suspension of PB (IV)	Daily (28 days)	ICPMS/AAS of spleen (+) Spleen weight (+) Histopathology (+)	Accumulation in spleen(S) Inflammation Degradation of erythrocytes/ lymphocytes << (S) Spleen weight >> (S)
Patra *et al.*, 2009	europium(III) hydroxide [EuIII(OH)3] nanorods	TE buffer/EuIII(OH)3 concentration of 1.25 mg kg-1/12.5 mg kg^{-1}/125 mg kg-1 (IP)	Daily (7 days)	ICPMS (+) Spleen weight (-) Histopathology (+)	Accumulation (NS) of mild follicular hyperplasia in spleen (NS)
Czubacka & Czerczak , 2019	PtNPs	PtNPs concentration of 10 mg kg^{-1} BW size 70 nm (IV)	Once	ICPMS (+) Spleen weight (-) Histopathology (-)	Accumulation of PtNPs in spleen (S)
Semete B *et al*, 2010	PLGA	polystyrene beads/4 mg PLGA (oral)	Daily (1, 7, 10 days)	ICPMS (+) Spleen weight (-) Histopathology/ Fluorescence (+)	Accumulation in spleen (NS) Histopathology (NS)
Singh *et al.,* 2013	MnO2-NPs*	Cyclophospamide/MnO concentration of 30/300/1000 mgkg–1 BW per day (oral)	Daily (28 days)	ICPMS (+) Spleen weight (-) Histopathology (+)	Accumulation of Mn(S) Splenic hyperplasia Inflammation Congestion in white pulp and red pulp (S)
Dumková , *et al.* 2017	PbO-NPs	1.23 × 106 particles/cm (IT)	Daily (6 weeks)	ICPMS (+) Spleen weight (-) Histopathology (+)	Accumulation of PbO(S) Histopathological features (NS).

Author	Nanoparticles	Dose	Duration of Administration	Spleen Tests	Results of Spleen Tests
Park *et al.*, 2017	Al-NPs, AlONPs, AlCeONPs	Water/AI-NPs concentration of 2 mg kg^{-1}/6 g kg^{-1} each type of AiNPs (oral)	Daily (28 days)	ICPMS (+) Spleen weight (-) Histopathology (+)	Accumulation of 3 types of AiNPs (S) Histopathology (NS)
Smulders *et al.,* 2015	Nano-silicon dioxide (SiO2)	Animal not exposed/00 mg SiO2 concentration of 50 ul (IT)	Once	ICPMS (+) Spleen weight (-) Histopathology (-)	Accumulation of SiO2 (S)
Shahbazi *et al.*, 2013	PSi NPs*	Concentration of 700 mg kg^{-1} (tail IV), TOPsi, TCPsi, APSTCPSi, THCPSi, UnTHCPSi	Once (1 day)	ICP-MS (+) Spleen weight (-) Histopathology (+)	Accumulation of 5 types of nanoparticles (S) Histological changes in APSTCPSi & UnTHCPSi (S)
Liu Y *et al.,* 2013	FITC-OCMCS	water/concentration of 500 ul (oral)	Daily (7 days)	ICP-MS (+) Spleen weight (-) Histopathology (+)	Low accumulation (NS) Histopathology (NS)
Janer *et al.,* 2014	TiO2NPs	water/TiO concentration of 100 mg kg^{-1} WB (oral)	Daily (1 day)	ICP-MS (+) Spleen weight Histopathology (-)	Accumulation (NS)

Effects of Nanoparticles on the Spleen. Nanoparticle technology offers great benefits to the field of healthcare, particularly for the role of nanoparticles as a drug delivery system. However, a number of reports indicate the presence of nanoparticle toxicity to the organs, including to the spleen especially discussed in this article. The toxicity of nanoparticles varies depending on the type, size, stability, and dose(10). Metal nanoparticles and inorganic nanoparticles are the two types that frequently cause toxicity, with a corresponding increase in the likelihood as the dose becomes higher(11). Unstable surface area of nanoparticles will trigger toxicity since such instability facilitates nanoparticle interaction with adjacent cells. The unstable surface area of the nanoparticles is caused by the nanoparticles having a high surface area. High surface area means more surface energy so it wants to share the energy with other sources. In addition, the size of nanoparticles greatly affects their distribution; the smaller the size, the easier it is for nanoparticles to penetrate a cell structure. This is because the larger nanoparticles sometimes stick to the outside of the membrane or stick to the surface and cause some distortion(4,12). Also, nanoparticles with higher solubility will have less difficulty in passing through the endothelial membrane or tight junctions. On the one hand, this has a positive effect when nanoparticles deliver drugs so that the drug delivery system is better. However, the ease with which small nanoparticles pass through the membrane can cause toxic side effects(3).

Gold Nanoparticles (AuNPs). AuNPs are a precious metal with valuable and potential beneficial properties for therapy as an anti-cancer but are still limited to in vivo rodent studies(13). Research found in this review that spherical AuNPs of size 10nm, 30nm, 60nm administered intraperitoneally and size 5, 10, 50 nm administered intravenously did not cause toxicity to the spleen. However, it needs attention to chronic administration, because it can accumulate in the spleen, although over time it decreases(14,15). Research outside the review shows a modification of the addition of surface layer AuNps showed no toxicity effect on spleen and AuNPs do not accumulate in spleen parenchyma, only on macrophage cells(16). The dose of nanoparticle administration determines the toxicity(17) as well as the shape and type of the surface layer of AuNPs(18). To obtain a holistic inference, more research on these nanoparticles is needed. Further studies on the toxicity of AuNPs in animal models of chronic disease should also be encouraged.

Copper Nanoparticles (CuONPs). CuONPs at concentrations of 100/200/500/1000 ug/kg body weight caused toxicity to the spleen and they are more toxic than herbs nanoparticles(6). Other studies have also shown toxicity to the spleen(19,20). However, review studies suggest that the toxicity of CuONPs may be attenuated by performing the adequate surface modification, size, dissolution factor, selection of exposure routes may decrease the risk of toxicity(21). CuONPs are inorganic nanomaterials frequently used in semiconductor devices, batteries, microelectronics, gas sensors, and other industries. The study results showed that oral administration of CuONPs can cause pathological changes not only in the spleen but also in the liver and kidney. In addition, CuONPs is toxic to mammalian reproductive organs[(22)]. Therefore, the environmental fate due to these nanoparticles must be carefully determined, and criteria for sustainable applications in various fields must be determined.

The mechanism for the toxicity of CuONPs lies in their action to increase TNF-α proinflammatory cytokines and reduce IL-10 anti-inflammatory cytokines. TNF-α cytokines stimulates cell apoptosis by initiating caspase-8, caspase-9, and caspase-3 of mitogen-activated protein kinase (MAPK) and downregulating pAkt and Bcl-2 as apoptosis suppressor genes. This inflammation causes damage or atrophy to the cells, resulting in reduced spleen weight [6,19]. However, review studies suggest that the toxicity of CuONPs may be attenuated by performing the adequate surface modification, size, dissolution factor, selection of exposure routes so make minimize toxicity[(21)].

Iron Nanoparticles (IONPs) & Supramagnetic Iron Nanoparticles (s-SPION). IONPs administered intravenously at concentrations of 1.5, 2.5, 5 mg Fe/kg did not cause toxicity to the spleen. This was indicated by the constant weight and histopathological features of the rat spleen which showed plasmacytosis, a condition not categorized as a sign of toxicity[(23)]. If IONPs accumulate, the macrophages can easily degrade and recycle them into varied Fe ions, including ferrous Fe (II) and ferric Fe (III), which are then reserved as iron stores in the body (haemoglobin, ferritin, transferrin). Similarly, s-SPION size 10 and 25 nm at a concentration of 90 mg Fe/kgBW administered intraperitoneally did not induce toxicity to the spleen[(24)]. However, another review of IONPs and s-SPION have reported teratogenic toxicity. Reports of toxicity still contradict the in vitro and in vivo findings and lead to different toxicities in different study models. There are many factors affect toxicity such as dose, concentration, time, surface chemistry, cell type, interaction media, internalization mode, which requires a very complicated and varied study[(25)].

Zinc Oxide Nanoparticles (ZnO-NPs). ZnO-NPs administered orally to rats that found in this review at concentrations of 100, 200, 300 mg kg^{-1} induced toxicity. This was shown by the accumulation of ZnO that increased correspondingly at greater concentrations in the spleen. In addition, a change in the spleen weight was found[(26)]. Toxicity develops as the presence of nanoparticles leads to electrolyte imbalances, such as in Na, K, Ca, P, and Mg. This condition disrupts the cell-cycle regulation, thus stimulating the formation of ROS and inducing oxidative stress in the spleen cells. Consequently, immunotoxicity manifests itself in the spleen as suppression of CD-86, CD-80, and CXCR2 expressions, which results in reduced chemokines for leukocytes and increased distribution of T cells in lymphocytes[(27)]. In vivo ZnOps toxicity studies have not been widely carried out. The results of this nanoparticle toxicity review in vitro demonstrated the presence of cytotoxicity, oxidative stress, and mitochondrial dysfunction. Therefore, the use of these nanoparticles may be directed to other benefits such as wastewater treatment. However, ecotoxicological studies are also needed[(28)].

Silver Nanoparticles (AgNPs). Study of AgNPs in this review caused spleen toxicity in the mice given AgNPs at concentrations of 0.0082, 0.0025, 0.074, 0.22, 0.67, 2.0, 6.0 mg kg^{-1} with size 20 nm and 100 nm. This was evidenced by nanoparticle accumulation, increased weight, and histopathological changes. The histopathological features showed inflammation and brownish colour (degradation of red blood cells/lymphocyte reduction) in the spleen tissue[(29)].

A study by Savery et al.[(29)] found the toxicity induced by silver nanoparticles (AgNPs). The immunotoxic effects of AgNPs appear as a decrease in the number of lymphocytes and decreased natural killer (NK) cell activity. AgNPs also become toxic when they induce oxidative stress in the endoplasmic reticulum of spleen cells. In addition, an elevated weight of the spleen indicates an accumulation of nanoparticles. A study by Wen et al.[(30)] suggested that AgNPs massively accumulated in the spleen increase the weight of this organ, thus leading to immunotoxicity in the form of unifocal necrosis of lymphocytes in the white pulp and increased multifocal macrophages shown in the histopathological features of the mouse spleen.

AgNPs are extensively used in medical devices due to their antimicrobial properties. Compared to other nanoparticles, AgNPs are more toxic to microbes. These nanoparticles are effective against gram-positive, gram-negative, and multi-drug resistance (MDR) bacteria[(31)]. However, the use of these nanoparticles still needs to be investigated because the results of in vivo toxicity review studies reported the accumulation and toxicity of Ag to the spleen and other organs, whether given by inhalation, intratracheal, intravenous, or oral in rodents[(32)].

Europium (III) Hydroxide [EuIII$(OH)_3$] Nanorods. Europium is known to have pro-angiogenic properties with the potential as an alternative therapy for patients with cardiovascular disease (CVD) and ischemic diseases as well as for wound healing. Other research suggested that these nanorods can intensify endothelial cell proliferation when administered in the 20-50 µg mL^{-1} concentration range. This pro-angiogenic mechanism results from the mitogen-activated protein kinase (MAPK) signalling pathway in the endothelial cells[33]. Although research is still limited in vitro, these nanoparticles are reported to cause a longer cell life span and prevent neurodegenerative diseases through autophagy[34].

EuIII$(OH)_3$ toxicity studies are very rare. The nanoparticles at concentrations of 1.25 mg kg^{-1}, 12.5 mg kg^{-1} and 125 mg kg^{-1} given intraperitoneally did not show any toxicity to the spleen[35]. The same results were reported by Bollu et al.[36] which stated that europium is non-toxic, however there is only moderate accumulation of Europium despite the high administration dose.

Platinum Nanoparticles (Pt-NPs). In vitro toxicity studies of HepG2 in acute exposures did not show toxicity, but in the long term, it may cause adverse effects[37]. Likewise, in cancer cells these nanoparticles are non-toxic at therapeutically relevant concentrations[38]). Another study of these nanoparticles induced cytotoxicity and apoptosis in CHANG and HuH-7 cells[39]. It also causes toxicity to algae[40]. Intravenously administered Pt-NPs in this review for mice at a concentration of 10 mg kg^{-1} body weight and a size of 70 nm did not cause spleen toxicity. These metal nanoparticles are harmless as a result of their rapid excretion and undetectability in plasma 24 hours after injection[41]. Unfortunately, the in vivo study of Pt-Np has acute toxic effects on cardiac electrophysiology and can induce threatening cardiac conduction blocks[42].

Poly(lactide-co-glycolide) Nanoparticles (PLGA). PLGA nanoparticles are more widely distributed in the liver and kidney instead of the spleen, however oral administration of 4 mg of PLGA to mice indicated no toxicity to the spleen[43]. Review studies related to the toxicity of PLGA in other organs are still controversial, some studies show the toxicity but others are the opposite[44]. These nanoparticles are commonly used to describe cancer conditions (cancer imaging) and cancer treatment. They are able to effortlessly penetrate the endosomal membranes and deliver drugs encapsulated in the cells and easy to eliminate by the macrophages in the reticuloendothelial system (RES), including in the spleen[45].

Manganese Oxide Nanoparticles (MnO_2-NPs). Administration of MnO at concentrations of 30, 300, and 1000 mg kg^{-1} body weight resulted in spleen toxicity. This was indicated by nanoparticle accumulation in the spleen and histopathological changes in the form of hyperplasia, white pulp and red pulp congestion, and spleen cell inflammation. The mechanism for toxicity of MnO^{2-}NPs takes place through induction of ROS that causes oxidative stress and corresponding damage to the DNA of the cells. As a result, the use of these nanoparticles should be carefully reviewed. Meanwhile, the widespread application of such nanoparticles today includes magnetic resonance imaging (MRI) contrast agent and drug delivery system[46]. In other organs such as the testis, subcutaneous injection (100 mg kg^{-1}) of MnO_2^- NPs to male Wistar rats caused a significant decrease in the number of sperms, spermatogonia, spermatocytes, diameter of seminiferous tubes and in the motility of sperms[47]. Fifty percent of rats have died when treated with high dose of MnO (500 mg / kgbw)[48].

Lead Oxide Nanoparticles (PbO-NPs). Instead of causing toxicity to the spleen, PbO-NPs are toxic to the rat liver by inducing hepatocyte hypertrophy, focal necrosis, and inflammation in some areas. In addition, lead oxide is able to easily penetrate the blood-brain barrier (BBB) and accumulate in the hippocampus and cortex of the rat brain, thus changing the morphology of the rat brain and damaging the central nervous system[49]. Lead is assumed to cause damage to the environment and the human body, and overexposure to lead can damage the central nervous system, haematology, kidney, and reproductive organs. The manifestations include abdominal pain, joint pain, constipation, anorexia, muscle pain, headache, decreased libido, sleep disorders, anaemia, nephropathy, encephalopathy, and seizures[50]. Another publication also states that lead nanoparticles are considered toxic and harmful to humans and environment. Currently, lead nanoparticles are more widely used for storage batteries, ceramics, and glass industry, although they are reported to have anticancer and anti-bacterial effects. However, due to their potential environmental damage, there is an urgent need to develop standardized test procedures to study the potential harm of these nanoparticles to human health and the environment[51].

Aluminium-based NPs (Al-NPs), Aluminium Oxide NPs (AlONPs), Spherical Aluminium Cerium Oxide NPs (AlCeONPs). Aluminium is categorized as a metal nanoparticle and commonly acts as a catalyst in industrial exhaust gas purification to prevent pollution as well as in cosmetics. With the strong, hard, wear resistant, and highly-biocompatible properties, aluminium nanoparticles can be used as teeth and bone implants[52]. Aluminium administered orally at concentrations of 2 mg kg^{-1} and 6 mg kg^{-1} did not show any toxicity to the spleen[27]. These nanoparticles accumulated in the spleen for only 24 hours after exposure, and instead of increasing ROS, they reduced excessive ROS formation. In contrast to other studies that showed aluminum nanoparticles doses of 15, 30 or 60 mg kg^{-1} body weight given to male Swiss albino mice for 5 days showed accumulation of aluminum in the spleen, brain, liver and kidneys and correlated with anatomical abnormalities, redox imbalance and DNA damage(53).

Nano-silicon Dioxide (SiO_2) & Porous Silicon Nanoparticles (PSi NPs). SiO_2 nanoparticles have been widely applied in different sectors, including agriculture, food, and cosmetics. Approximately 1.5 million cans of SiONPs were sold in the black market in 2012. Data of intra-tracheal administration of 100 mg SiO_2 to rats did not show any toxicity to the spleen(54). However, another study showed that intravenous administration of SiNPs at 10 or 30 mg kg^{-1} showed an increase in megakaryocytes in the spleen(55) and doses up to 20 mg kg^{-1} cause DNA damage in the spleen(56). Likewise, in vitro studies showed that silica nanoparticles cause much oxidative stress and cell apoptosis in culture(57). APSTCPSi and UnTHCPSi types of Psi-NPs administered intravenously at a concentration of 700 mg kg^{-1} caused toxicity to the spleen(58). These nanoparticles increase ROS production and easily interact with the immune system, leading to immunotoxicity marked by a decreased number of T cells and macrophages, particularly in the spleen. Consequently, there is shrinkage of splenic white pulp(59). Porous silicon is widely used in the medical field for diagnosis and therapy since porous silicon nanoparticles have more valuable properties for biomedical applications compared to other nanoparticles.

Fluorescein Isothiocyanate Oleoyl-carboxymethyl-chitosan Nanoparticle (FITC-OCMCS). Chitosan acts as a carrier in polymer nanoparticles for drug delivery through various routes of administration(60). Chitosan-modified OCMCS increases the permeability of macromolecular drugs to cross the epithelium. OCMCS is a non-toxic polymeric nanoparticles that have proved to cause no toxicity to the spleen, liver, kidney, and heart when administered orally(61). Although in general chitosan nanoparticles do not cause toxicity, further research remains a concern because there are many variations of chitosan nanoparticle preparations, both modifications for drug delivery, namely therapeutic proteins including polyanions derived from nature and modification of grafting of the hydrophobic part to the polysaccharide chain(62). A review of chitosan nanoparticles reported disturbances in embryo development and neurobehavior toxicity(63).

Titanium Dioxide Nanoparticles (TiO_2NPs). TiO_2 nanoparticles are bright and therefore suitable as a pigment. TiO_2 is frequently used as a base for color paint, plastics, paper, ink, medicines, food products, and toothpaste, a skin whitening pigment, and a base for sunscreen ingredients. Single oral administration of TiO_2 at a concentration of 100 mg kg^{-1} did not cause any toxicity to the spleen(64). In another study, however, chronic administration can lead to spleen toxicity and reduce immunity(65). Intratracheal administration/ inhalation of TiO_2 can also cause lung inflammation. The intraperitoneally administered TiO_2 will damage the liver, kidney, and myocardium(66). Moreover, According to the International Agency for Research on Cancer, titanium dioxide nanoparticles cause cancer in humans(67).

Although these nanoparticles pose a risk of toxicity, their physicochemical properties are unique and have great therapeutic potential. Evidence shows that synthesizing TiO_2-NPs through biological methods is safer than synthesizing through chemistry or physics. Green synthesis using biological methods is environmentally friendly, efficient, simple, safe, and cost-effective on a large scale. However, the most difficult challenge in the biosynthesis of TiO_2 nanoparticles from biological materials is contamination from biological cells, which can have adverse effects in biomedical applications. Therefore, further research is needed to increase the stability of nanoparticles in synthesizing these nanoparticles(68).

Limitation. This scoping review has limitations. First, the literature search was conducted only via Pubmed/ MEDLINE, so the scope was narrower. Nonetheless, it is a trusted medical journal search engine and contains more than 22 million biomedical published articles. Second, this review only takes animal studies and spleen organ toxicity, due to the limited evidence of spleen damage in studies involving humans. In the future, the collection of toxicity test data on all organs (not only the spleen) after the administration of nanoparticles becomes very important. Search for articles with broader search engines other than PubMed such as Google Scholar citation is still needed.

CONCLUSION

Thirteen nanoparticles induce toxicity in rodent spleen in the form of accumulation in the spleen and/or damage seen from the histopathology of the spleen. Only four nanoparticles had no toxic effect on the spleen. Although Titanium dioxide has no toxicity effect on the spleen, it does show toxicity effects on other organs. Metal nanoparticles generally have a toxic effect. Toxic effects depend on the dose, size of nanoparticles, route of administration, and method of synthesis.

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