INTRODUCTION

The existence of a protective "sanctuary site" in the testis, which limits drug concentration or sequestration, has been suggested. This sanctuary site is typified by the testis, an essential compartmentalized reproductive organ within the scrotum. It is encapsulated in the outermost to the innermost thick layers of connective tissue capsules, tunica vaginalis, tunica albuginea, and tunica vasculosa. Septa from the tunica albuginea partition the testis into different lobules. Each of these lobules contains seminiferous tubules that are approximately 200 μm in diameter, with a total length of ~600 m that contributes to about 60 percent of the total volume of the testis.

Nanoparticle delivery system, highly active antiretroviral therapy, and testicular morphology: The role of stereology

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Abstract

The conjugation of nanoparticles (NPs) with antiretroviral drugs is a drug delivery approach with great potential for managing HIV infections. Despite their promise, recent studies have highlighted the toxic effects of nanoparticles on testicular tissue and their impact on sperm morphology. This review explores the role of stereological techniques in assessing the testicular morphology in highly active antiretroviral therapy (HAART) when a nanoparticle drug delivery system is used. Also, NPs penetration and pharmacokinetics concerning the testicular tissue and blood–testis barrier form the vital part of this review. More so, various classes of NPs employed in biomedical and clinical research to deliver antiretroviral drugs were thoroughly discussed. In addition, considerations for minimizing nanoparticle-drugs toxicity, ensuring enhanced permeability of nanoparticles, maximizing drug efficacy, ensuring adequate bioavailability, and formulation of HAART-NPs fabrication are well discussed.

KEYWORDS

blood–testis barrier, highly active retroviral therapy, nanoparticles, spermatogenesis, stereology, testis
Spermatogenesis occurs in this guarded region of the seminiferous tubules that is compartmentalized by the blood-testis barrier (BTB). This compartmentalization presents a boundary between the area of spermatogenic cells and the vascular environment, which provides an enabling environment for spermatogenesis.

The testis has been described as an organ that may harbor human immunodeficiency virus 1 (HIV-1). Several studies have reported adverse effects of antiretroviral drugs (ARVDs) on the reproductive parameters, suggesting that appropriate consideration has not been given to the overall effects of highly active antiretroviral therapy on the testis. Deleterious effects of highly active antiretroviral therapy on sperm motility continue to add to the debate. On the one hand, studies have reported no changes in semen parameters of the HIV-1 patients undergoing highly active antiretroviral therapy (HAART). On the other hand, changes in semen parameters of HIV-1 patients under HAART have been suggested, with likely adverse effects of HAART on the reproductive organs. This includes a significant reduction in total sperm motility in the groups of animals treated by HAART, with lamivudine, nevirapine, and zidovudine. Together these reports have established that HAART penetrates the seminiferous tubules but in reduced quantities because of the blood-testis barrier (BTB). However, HAART has been effective at improving Cell of Differentiation 4 (CD4) counts, suppressing viral replication, and viral load to undetectable levels in many patients.

The BTB that partitions the seminiferous tubules and the vascular compartment of the testis significantly reduces the uptake of ARVDs into the testis. This reduction reflects the action of the breast cancer resistance protein (BCRP) and efflux transporters P-glycoprotein (P-gp) that together block and restrict the penetration of ARVDs. However, difficulty in penetration of BTB can be overcome by loading ARVDs with nanoparticles. A few studies have reported penetration of nano formulated ARVDs across the BTB. Accumulation of lopinavir, ritonavir, and efavirenz coupled with poly(lactic-co-glycolic acid) (PLGA) nanoparticles have been shown in peripheral blood mononuclear cells in mice testis for 28 days without cytotoxicity.

2 | NANO PARTICLE PENETRATION IN HAART FROM BENCH TO CLINIC

Despite the technological advancement in medical diagnosis and treatment, the toll of infectious and noncommunicable diseases is still high. There is a need for simple, inexpensive, rapid, and sensitive point-of-care diagnostic tools and drug therapies with reduced toxicity and side effects to minimize mortality. Nanoparticles with unique properties are being incorporated into many products as the horizon, and commercial interest in nanomedicine is broadening. Over 500 consumer products in the market claim to contain elements of nanoparticles and more are still emerging. This uncontrolled use tends to increase human exposure to nanomaterials.

Characterization protocols, predictive toxicities, and hazard capabilities of nanodevices and nanomaterials need to be validated. Nanomaterials have been attractive for technology development in the basic sciences and have been used in medicine. Nanotechnology synthesis and the use of the ultramicroscopic particles invisible to the unaided eye are not a latter-day invention from which nanomedicine arose. This area embraces an increasing number of miniaturized technology platforms as they are adopted in biomedicine to solve medical problems. It has the potential to completely shape, direct, and change the future of medical treatments over the next decade.

2.1 | Classes of nanoparticles

Nanoparticles (NPs) are materials in the order of approximately 100 nm, similar to the size of HIV particles. There are several routes by which NPs can enter the organs and bloodstream, with inhalation being one of the more accessible routes. A large number of NPs are safe with beneficial effects, however, cases of toxicities have also been documented for some NPs. Nanoparticles are classified into different categories based on their properties and diverse application. These include metal nanoparticles (MNPs), semiconductor nanoparticles (SCNPs), ceramic-based nanoparticles (CBNPs), polymeric nanoparticles (PNPs), carbon-based nanoparticles (CNPs), and lipid-based nanoparticles (LBNPs) (Figure 1).

Metal nanoparticles have received significant attention possessing optical and electrical characteristics with clinical and medical applications. Their absorption and storage of a large number of electrons, quantum detention ability, large area energies, and large surface area to volume ratio are the characteristics that have made silver, gold, zinc, cadmium, platinum, copper, and iron popular for use in the synthesis of nanoparticles. Owing to their physicochemical attributes, MNPs derived from silver, gold, and copper are being developed as drug carriers for use in the diagnosis, treatment, and bioimaging.

Semiconductor nanoparticles are derivatives of elements, compounds, or a combination of two or more elements that appear in groups IV and VI in the periodic table between metals and nonmetals.
Semiconductor nanoparticles, such as silicon (SiNPs), germanium (GeNPs), tin (SnNPs), selenium (SeNPs), tellurium (TeNPs), zinc oxide (ZnO), zinc sulfide (ZnS), cadmium sulfide (CdS), cadmium selenide (CdSe), and gallium nitride (GaN) are used in the area of electrical, optical, electronic, and fiber networks.\(^{33}\)

Ceramic-based nanoparticles are inorganic nonmetal solids of different forms; amorphous, porous, and polycrystalline.\(^{34}\) These NPs are used in medical imaging, photo catalyzes, and photodegradation of dyes.\(^{35}\) Also, CBNPs such as titanium dioxide (TiO\(_2\)) and aluminum oxide (Al\(_2\)O\(_3\)) has been widely used in the manufacturing of nano delivery systems,\(^{36}\) with silica, albumin, and iron oxide being employed in drug delivery systems.\(^{37,38}\)

Graphite, graphene, nanodiamonds, carbon nanotubes, and Buckminsterfullerene (C\(_{60}\)) are the most widely employed CNPs.\(^{39}\) Some of these CNPs can form carbon nanotubes,\(^{40}\) including graphene\(^{41}\) that have been used in therapeutic and drug delivery systems or as cellular labeling agents.\(^{40-42}\) The therapeutic application of Buckminsterfullerene, as an anti-HIV agent, has been reported.\(^{43}\)

Polymeric nanoparticles (PNPs) are organic-based NPs with solid mass wrapped within a particle.\(^{44}\) Polymers are distinct because of their huge molecular structures, crystallization performance, long-chain involvement, and glass transition.\(^{45}\) Application of carboxy-terminated poly(D,L-lactic-co-glycolide)-block-poly (ethylene glycol) (PLGA-b-PEG-COOH) and poly(D,L-lactide-co-glycolide)/montmorillonite (PLGA/MMT) PNPs are used in drug delivery systems.\(^{46,47}\)

Lipid-based nanoparticles (LBNPs) contain functional lipids that make them ultimately tolerated and degraded to a nontoxic precipitate. Over a decade, LBNPs such as ethosomes, lipid nanoemulsions (LNE), liposomes, transfersomes, solid lipid nanoparticles (SLNs), and niosomes have received broad attention for the effectiveness and safety in drug delivery systems.\(^{48,49}\) Furthermore, high thermal stability, ease of prepare, biocompatibility, large-scale preparation, cost-effectiveness, biodegradability, and robust loading capacity are the advantages of LBNPs.\(^{50,51}\)

### 2.2 | Application of nanoparticles in medicine

The emerging field of nanotechnology may change the contemporary treatment modality of HIV by enhancing the delivery of highly active antiretroviral drugs to the intended organs and their effectiveness.\(^{52-56}\) This novel direction has been credited to the application of various NPs, with the ability to penetrate the blood-testis barrier.\(^{57}\) Several studies have described the intracellular drug delivery system using NPs: receptor-mediated phagocytosis of nanocarriers, passive diffusion of free drugs, nonspecific phagocytosis of nanocarriers, and pinocytosis process of nanocarrier uptake as pivotal mechanisms of action.

There are intracellular drug delivery systems that may employ a combination of more than one mechanism. The drug may be broken down, leading to an ineffective treatment when the NP is released within the lysosome. However, effective treatment can be achieved when drugs are released within the cytosol.\(^{58-60}\) Testis CD68\(^{+}\) macrophages are indulgent to immunodeficiency virus-1 infection and aid replication of the virus without affecting testosterone secretion.\(^{61}\)

The previous study has indicated that the degree to which antiretroviral drugs can penetrate anatomical compartments, anatomical
sanctuary regions, and viral reservoir sites is based on the changing interaction between metabolism, drug efflux, and influx. These have been attributed to unproductive viral suppression, viral persistence, and the virus’s resistance to anti-viral drugs.62 There are increasing pieces of evidence that ATP-binding cassette transporters (ABC) are one of the essential factors that impede the entrance of drugs into the testes, moreover, studies have demonstrated that testes could retard the entrance of antiretroviral drugs and act as a harbor for HIV-1, thereby causing persistent HIV-1 infections and subsequent drug resistance.7

A previous study revealed that creating an equilibrium between the efficacy, safety, permissibility, and administration of antiretroviral drugs are essential factors that require maximum attention in achieving a good outcome in the management of HIV infections.63 However, aspersions have been cast on these antiretroviral drugs, especially the nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), owing to reported toxicities, side effects, and adverse effects.2 Several studies have linked highly active antiretroviral therapy (HAART), especially nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI), to insulin resistance and possible cause of diabetes mellitus in HIV-infected persons.64-66 In the same vein, gastrointestinal disorder and lipodystrophy syndrome have been described.57 Another profound adverse effect documented is cardiovascular and liver toxicities68,69 and severe hyperbilirubinemia.70 These adverse effects have resulted in drug noncompliance with the patients, changes in therapeutic modalities, and discontinuation of treatment.71

Tissue-specific drug-targeted methods have proven to boost the drug’s effectiveness at a low dose while reducing adverse effects by controlling the bio-distribution of the drug in nonspecific tissues.72-74 Suppression of the viral load to an undetectable level and minimizing antiretrovirals’ toxicity without affecting the therapeutic concentration has been described as the primary goal in the management of HIV infection. In this regard, the usefulness of antiretroviral drug-loaded NPs has received considerable attention. Notably, Ochekpe et al75 have described the application of nano-technology to HIV therapy as a core area in drug delivery systems that addresses the issue of bioavailability, tissue distribution, drug level imbalance, and minimizing toxic effects of common antiretroviral drugs.76 Likewise, antiretroviral drug-loaded NPs have been delineated as a drug delivery system that ensures an improvement in side-effects of antiretroviral drugs.77

Nanoparticle-loaded drugs hold a promising future in nanotherapeutics because of their ability to penetrate biological membranes.52 Nanomaterials have received a wide range of interest, and applications have increased in drug delivery systems to reduce drug adverse effects and toxicities. Priority has been placed on synthesized NPs to achieve a wide range of applications in the field of nanomedicine. Still, not all nanoparticles can be used in this regard due to the regulations of the Royal Society and Royal Academy of Engineering.77 Different characteristics that make these NPs highly applicable include their capacity to absorb and pick up other molecules, their quantum characteristics, and more substantial surface to mass ratio, which proved to be larger than other particles. This larger surface primarily enables NPs to adsorb, bind, and pick up other substances such as proteins and drugs.78

The method of NP synthesis also plays a role in the toxicity of antiretroviral drug-loaded NPs. There are two approaches to manufacturing NPs; one in which bulk products are curtailed, which is known as the top-down approach, and one in which materials are combined to form larger particles known as the bottom-up approach.79 Physical, chemical, and green synthesis of NPs have previously been discussed. Green synthesis methods have received a wide range of attention than physical and chemical processes because of their natural stabilizing and reducing abilities. Consequently, there is more interest in the biosynthesis method of NPs employing microorganisms nowadays.80

To maintain environmentally safe procedures, the use of chemicals that usually come with hazards should be abolished, whereas green synthesis processes that present biological methods, irradiation methods, polysaccharides, and blended-valence polyoxometalates should be embraced. Moreover, the green synthesis offers enormous benefits compared to procedures that require chemicals linked to ecological hazards should be embraced. Choosing a solvent and environmentally safe stabilizing and reducing agents free of hazards must receive special consideration during the green manufacturing of NPs.81,82 The previous study has revealed that steady release and effective therapeutic drug delivery of NPs and materials depend on their synthesis method.83

To date, only a few studies have investigated the penetration of antiretroviral drug-loaded NPs or HAART through the blood-testis barrier. The previous research has reported the distribution and accumulation of nano-coupled antiretroviral drugs such as lopinavir, ritonavir, and efavirenz-loaded poly lactic-co-glycolic acid nanoparticles in the testes of mice.71 This result indicates the need to utilize NPs for delivering antiretroviral drugs into the male reproductive system.

There have been tremendous efforts to formulate HAART NPs against a wide range of HIV-1 strains, but the issue of toxicity resulting in DNA damage has been reported.52 Few NPs, such as polymeric, liposomes, silver/gold, have been reported to enhance the delivery of antiretroviral drugs effectively to combat or treat HIV infection.84 Ritonavir, lopinavir, and efavirenz coupled with PLGA NPs21 and dapivirine coupled with poly (ε-caprolactone) NPs85 have been reported to be in the preclinical stage amongst other nano formulated HAARTs.

3 | PHARMACOKINETICS OF NANOPARTICLES IN RELATION TO TESTICULAR TISSUE AND BLOOD–TESTIS BARRIER

The pharmacokinetics (absorption, distribution, metabolism, and excretion) of NPs largely rely on their physiochemical characteristics.86
Nanoparticles presented in solid or liquid forms also penetrate the barriers as well as associated physical, biological, and chemical processes of the tract, consequently, altering and transforming their pharmacological and toxicological properties. Importantly, particle size is an essential factor, the smaller the particle size, the more effective its disease curative effects. As a result, NPs synthesized drugs have higher penetration, proper absorption, more extensive distribution, better metabolism, and greater bioavailability compared to drugs of the same size.

Nanoparticles are administered in various ways, including oral, percutaneous, pulmonary, nasal, and injection. Following administration, NPs are absorbed into the circulatory system and get excreted via feces or other means. The mucosal lining and epithelial tissue of the gastrointestinal tract have been identified as primary barriers to the absorption of nano-synthesized drugs. Previous studies show NPs to be absorbed through intestinal enterocytes. More so, the Peyer's patches at the small intestine wall are the site of absorption for NPs within the range of 50–200 mm.

Several animal studies have documented different types of NP absorption; through the skin, by the skin through lymph nodes and the lymphatic system, through the olfactory region (nasal), which goes straight to the central nervous system, a perfect choice for crossing the BTB. Furthermore, the inhalation method whereby NPs are absorbed through alveoli has been reported to be one of the best methods because of the larger surface area of alveoli which permits easy ingress of NPs to the lymphatic and blood circulation system. Additionally, when considering drug response and bioavailability, various injectable methods of NPs should be employed.

The significant benefit of the biodistribution of NPs is the ability to determine the likely mechanism of action of NPs. After absorption, effective NP distribution depends on the composition, size, morphology, surface charge, and coating effects. Based on composition, there was reported evidence of a greater affinity of mesoporous silica NPs to the lungs than polymeric NPs to the liver. Regarding the size, for NPs to bypass the liver hepatocytes, it must be smaller. The justification for this is based on the reduced blood circulation period due to bigger particles being taken up by the spleen and the liver.

Coating NPs with starch-like materials such as dextran, polyethylene, and other coating materials predominantly intensifies the biodistribution of the NPs. Degraded products of biodegradable NPs are simply metabolized, whereas the metabolism of metal NPs such as silica, silver, iron oxide, and gold is intricate. For instance, a previous study reported that a quantum dot NP remained in the body for two years. Further, one of the brain’s supporting cells, the astrocytes, has been identified as the site of metabolism for iron oxide.

There are several elimination methods of NPs and drugs, but the primary process is renal excretion, which is a multiplex method that involves glomerular filtration and tubular secretion. Interestingly, there has been a significant link between drug pharmacokinetics and drug transporters located at the junctions of the BTB. A recent study reported the drug transporters that are in different regions and junctions of the BTB are the determinants of the number of drugs and chemical agents that enter the testis under healthy and disease conditions. The BTB is a unique blood barrier in the body because of additional and specialized barriers. Besides the tight junction (TJ) and gap junction (GJ) that are also found in other barriers, the BTB also contains the adherens junction (AJ), ectoplasmic specialization (ES), desmosome, hemidesmosome, and tubulobulbar complex (TBC).

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**FIGURE 2** The Blood-Testis Barrier and the Nanoparticle penetration

The hypothesis of how nano-Ag penetrates the blood-testis barrier. (A) The outline of part of the seminiferous tubule. (B) only depicts the Sertoli cells which contact with the basal lamina viewed from the outside of seminiferous tubules to the inside of seminiferous tubules.
The BTB separates the adluminal and basal compartments of seminiferous tubules so that sperm production can occur in the apical compartment posterior to the BTB without any interference. A previous study has reported the immunomodulatory function of the BTB based on preventing the formation of molecules or abnormal antibodies that may hinder sperm formation.\textsuperscript{109} The BTB provides an enabling and healthy environment for sperm production.\textsuperscript{110}

This barrier is made up primarily of the tight junctions between the Sertoli-Sertoli cells and actin-formed adherens junctions and a cytoskeleton-based junction, majorly the intermediate filament-forming desmosome junctions. Out of the three compartments in the substance of seminiferous tubules (basal, luminal, and adluminal), the BTB formed the demarcation between the adluminal and basal compartments. These compartments have been reported to be essential for the development of sex cells and protect them from NPs, foreign bodies, hormonal imbalances, toxins, and infectious diseases to perpetuate their reproductive function.\textsuperscript{111}

The seminiferous tubules, which is the testis’ functional unit, are surrounded by myoid cells, a contractile cell that propels the mature sperms from secretion, the seminiferous tubule into the epididymis, whereby it will mature. Nevertheless, the BTB starts with Sertoli cells, an epithelium covering the innermost part of the seminiferous tubule that functions to anchor and supply necessary nutrients during sperm formation.\textsuperscript{112-114}

Nanoparticles in the real sense and hypothetically cannot pass through the BTB. Still, some studies on animal models revealed that some characteristics of NPs allow penetration through the BTB, whereas larger particles do not penetrate\textsuperscript{115} (Figure 2). Wang et al\textsuperscript{116} reported that NPs could penetrate the BTB.\textsuperscript{116} A similar study that examined silver NPs revealed that small-sized NPs possess the capacity to penetrate the BTB, whereas large-sized NPs do not.\textsuperscript{117}

There have been contradictory reports on the ability of NPs to reach the testis and alter spermatogenesis. In a microscopic study, NPs were not found in the testis.\textsuperscript{118} Another study reported the ability of NPs to reach the testis,\textsuperscript{119} penetrate the BTB, and alter the process of sperm production.\textsuperscript{120} In a different study, the nontoxic effects of NPs on spermatogenesis were documented.\textsuperscript{121} Importantly, results from previous studies revealed that a small quantity of NPs is getting to the substance of testis irrespective of the method of administration\textsuperscript{115,122,123,124} (Figure 2). The unique characteristics of the BTB and NPs may complicate the conventional way of evaluating cytotoxicity and the effectiveness of NPs.\textsuperscript{125}

Although significant progress has been made on NP penetration across the testicular tissue. However, specific concerns have yet to be addressed regarding employing NP drug delivery systems in basic and clinical research, such as NP toxicity\textsuperscript{126} and the type and properties of NPs to be used. A previous study by Papageorgiou et al.\textsuperscript{127} reported that properties of NPs, such as surface features, crystalline properties, size, and chemical constituents, determine the toxicity profile of these NPs. The synthesis route is another issue that must be addressed when employing NPs in drug delivery systems. The biogenic bottom-up synthesis method has been viewed as a better method because of its viability and lack of toxicity, as reported by recent studies.\textsuperscript{128} The process of loading NPs with drugs must also be addressed when using NPs in drug delivery systems. Addressing the penetration of NPs in each of the junctions that constitute the BTB requires thorough investigation.

4 | NANOPARTICLES/ NANOFORMULATIONS USED IN CLINICAL RESEARCH

To date, only a few of these NPs have been approved for clinical use, whereas many are still in the pipeline of getting approval. Some of the NPs used in treating cancer, iron-replacement, bacterial and fungal treatments have been approved by the Food & Drug Administration (FDA) and the European Medical Agency (EMA). The important NPs used in clinical diagnosis and therapeutics are classified into two categories, namely, organic NPs, which include liposomal NPs, protein-based NPs, and polymeric NPs, and inorganic NPs, which include metal and metal oxide NPs.\textsuperscript{129}

The inorganic NPs have been successful in preclinical research. Iron oxide NPs have been developed and approved to treat anemia and imaging applications.\textsuperscript{130,131} Organic NPs, such as liposomes, have been an enormous success and have also been developed into vaccines, anesthetics, and fungal treatments.\textsuperscript{1,132}

Nanoparticles have been successfully employed with anti-cancer drugs to ensure the effective management of cancer. Doxorubicin, an anti-cancer drug loaded with pegylated liposomal HCl (CAELYX/ Doxil) was formulated and employed in metastatic breast cancer phase III clinical research.\textsuperscript{71} In another clinical research on heart disease, gold NPs were able to deliver drugs to telomerase and consequently alter cancer cells’ proliferation.\textsuperscript{133} In recent experimental research on the treatment of heart disease, it was evident that gold NPs loaded with Levosimendan (Simdax) and gold NPs with size 30 nm exhibited remarkable cardioprotective results in doxorubicin-induced heart failure rats, considerably better than rats treated with Levosimendan (Simdax) alone.\textsuperscript{134}

Liposomes were the first nanof ormulations approved by the FDA for clinical trials. The liposomal formulations such as doxorubicin and amphoterin B approval started in the mid-1990.\textsuperscript{135} Recently, Onivyde (liposomal irinotecan) was approved as a second-line treatment for metastatic pancreatic cancer. Marqibo (liposomal vincristine) was also recently approved for the treatment of pancreatic cancer, multiple sclerosis, fungi infections, and respiratory distress syndrome.\textsuperscript{37,136} There is clear evidence that liposomal formulations have become clinically stable and improved in nanotechnology, therefore, nanomedicines’ evolution remains relevant.\textsuperscript{37}

Polymer nanof ormulations such as Coagulation factor IX (Rebinyn) and Antihemophilic factor VIII (Adynovate) have also been investigated and approved for the treatment of hemophilia due to their more excellent protein stability and long half-life.\textsuperscript{137} Recently, Oncasar (pegaspargase) was approved for the treatment of conditions such as chronic gut, hepatitis, multiple sclerosis,
prostate cancer, among others. Protein nanoformulations such as Abraxane (albumin-bound paclitaxel) and Ontak (denileukin diftitox) have recently been approved to treat breast cancer, pancreatic cancer, and cutaneous T-cell lymphoma due to their more excellent stability, increased delivery to the tumor, and targeted T-cell specificity and lysosomal escape. The FDA receives new nanoformulations for clinical investigations yearly, and many have been approved for clinical use. As of October 2017, 56 clinical trials nanoformulations have been received or are in the inactive stage (Clinical Trials.gov).

Iron oxide nano-drugs such as Venofer Ferleccit have been studied extensively in the clinical trial phase. The FDA has approved them as an indication for iron replacement therapies. However, iron oxide nanoformulations used as a contrast enhancer reagent for magnetic resonance imaging are still in the clinical trial stage.

Several NPs have been clinically proven for the treatment of HIV/AIDS. The DermaVir patch was employed for immunotherapy of HIV/AIDS after being proven safe and tolerable in preclinical and phase I clinical trials and have consequently progressed to stage II trials. An L-lysine dendrimer is in phase I/II trials. Silver NPs, Silver nanoparticles, silver nanoparticles, and PGLA NPs are all preclinical trials.

The first long-acting regimen of antiretroviral drugs, cabotegravir, and rilpivirine, has been approved to treat HIV. Recently, the role of nanoformulation of the long-acting injectable cabotegravir and rilpivirine on the treatment of HIV infection has been reported. Emphasis was placed on its advantages, such as reducing the number of drugs, minimizing drug-associated toxicity, reducing adverse drug effects, and treatment simplification. In another study, myristoylated cabotegravir produg was formed, and this crystal was formulated into nanoparticles. The nano myristoylated cabotegravir (NMCAB) that was fabricated has proven to improve biodistribution and viral clearance profiles in mice.

There is no unanimous consensus about toxicity or risks in most NPs used for clinical trials or nanomaterials developed for commercial purposes. Although biomedical researchers have made tremendous efforts to investigate the toxicological profile of these NPs, the results have not been convincing enough. Recently, organ toxicities of NPs have been highly documented. Previously, a study that examined toxicity in mice following chronic oral administration of CeO2 NPs, testis impairment, sperm DNA damage, sperm malformation, asthenospermia, and reduction in testicular cytology was reported. Nephrotoxicity, chronic cardiac toxicity, and other organ toxicities of NPs are summarized in Table 3.

5 | TOXICITY PROFILE OF ANTIRETROVIRAL DRUGS/HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AND NANOPARTICLES

Diverse works of literature have documented different toxicities of antiretroviral drugs ranging from mild to severe adverse effects on major organs and systems of the body. Consequently, the World Health Organisation has revealed that it has now become more difficult to distinguish the adverse effects of antiretroviral drugs from common complications of HIV infection. Despite numerous beneficial effects of HAART, research has unveiled toxicities, side effects, and even clinical adverse events. HAART is associated with clinical adverse effects such as hyperglycemia, gastrointestinal, and lipodystrophy symptoms.

The combination of different antiretroviral agents (HIV-HAART) exposes the entire body to multiple doses at high doses, resulting in enormous side effects, limiting the therapeutic effect, or resulting in toxicity. Numerous adverse effects of antiretroviral drugs on the organs and systems of the body have been documented; suppression of bone marrow, which would later result in thrombocytopenia and anemia, has been linked to the zidovudine, azidothymidine, tenofovir disoproxil fumarate, efavirenz, lamivudine, and stavudine. The previous study has reported peripheral neuropathy, lactic acidosis, hyperlipidemia, and insulin resistance as adverse effects of stavudine, which corresponds with other reports in Table 1. Renal dysfunction and nephrotoxicity have been linked with Nevirapine, Efavirenz, Stavudine, and Indinavir as shown in Table 1.

Despite the growing knowledge on the effects of HAART on male reproduction, there are contradictory findings concerning real sperm functional tests. Onanuga et al. (2018) reported a severe histological alteration of the seminiferous tubule in the experimental animals subjected to diabetes and HAART, although it is not clear whether the alteration was caused by drug-induced diabetes. Other studies have reported toxicity and adverse effects of antiretroviral drugs (Table 1), antiretroviral drugs coupled with NPs (Table 2), and toxicity of these nanomaterials on several organs (Table 3). Nevertheless, the NP drug delivery system has improved the efficiency of the delivery of antiretroviral drugs (such as Saquinavir) as well as a combination of different antiretroviral drugs. Gold and silver have been reported to have antiviral properties against a wide range of HIV-1 strains but posed high toxicity issues resulting in DNA damage and cellular apoptosis. Studies have shown that antiretroviral drug-loaded NPs or nanocarriers achieve adequate drug distribution to specific sites in the body and recognize HIV-infected cells and can deliver multiple therapeutic doses, thereby increasing drug efficacy.

Recently, toxicity has been reported in some of the antiretroviral drug-loaded NPs, as shown in Table 2. Madugulla et al. reported a significant decrease in litter size through the oral administration of lactoferrin NPs, however, there was no significant difference in the litter size and postnatal development of the same drugs administered through the vaginal route. This result could suggest that the toxicity of NP-loaded drugs may depend on the route of administration. Additionally, Ogunwuyi et al. reported that antiretroviral drug-loaded NPs (Nevirapine, Raltegravir, Zidovudine, and Lamivudine) are effective in the inhibition of HIV-1 infection in CEM T cells and PBMCs but are toxic at higher concentrations.
### TABLE 1  Toxicity profile of non-nano antiretroviral drugs

| S/N | ARDS | Studies                                                                 | Toxic effects                                      |
|-----|------|-------------------------------------------------------------------------|----------------------------------------------------|
| 1.  | Nevirapine | Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents | Hepatic necrosis<sup>155</sup>                     |
|     |      | Safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection | Hypersensitivity<sup>166</sup>                     |
|     |      | Limitations to treatment safety and efficacy: adverse effects of antiretroviral agents | Renal dysfunction<sup>167</sup>                    |
| 2.  | Efavirenz | A randomized cross-over study to compare raltegravir and efavirenz | Persistent and troubling neuropsychiatric symptoms<sup>168</sup> |
|     |      | A phase IV, double-blind, multicenter, randomized, placebo-controlled, pilot study to assess the feasibility of switching individuals receiving efavirenz with continuing central nervous system adverse events to etravirine | [169]                                               |
|     |      | Neuropsychiatric side effects of efavirenz therapy                         | [170]                                               |
|     |      | Acute Liver Toxicity due to Efavirenz/Emtricitabine/Tenofovir            | Hepatotoxicity<sup>171</sup>                      |
|     |      | CYP2B6 haplotype and biological factors responsible for hepatotoxicity in HIV-infected patients receiving efavirenz-based antiretroviral therapy | [172]                                               |
|     |      | EFV/FTC/TDF-associated hepatotoxicity: a case report and review           | [173]                                               |
|     |      | Hepatotoxicity in patients prescribed efavirenz or nevirapine             | Teratogenicity<sup>174</sup>                      |
|     |      | Periconceptional exposure to efavirenz and neural tube defects           | [175]                                               |
|     |      | Myelomeningocele in a child with intrauterine exposure to efavirenz       |                                                    |
| 3.  | Raltegravir | Severe rhabdomyolysis associated with raltegravir use                     | Skeletal muscle toxicity, Rhabdomyolysis, and Elevated serum creatine kinase (CK)<sup>176</sup> |
| 4.  | Zidovudine, or azidothymidine | Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access | Anemia, neutropenia and, more rarely, thrombocytopenia<sup>157</sup> |
|     |      | Management of the Adverse Effects of Antiretroviral Therapy and Medication Adherence | Bone marrow suppression<sup>177,178</sup>          |
|     |      | Tenoforv DF, emtricitabine, and efavirenz versus zidovudine, lamivudine, and efavirenz for HIV | Hyperlipidemia<sup>179,180</sup>                   |
|     |      | Improvement of dyslipidemia in patients switching from stavudine to tenofovir: preliminary results |                                                    |
|     |      | Lipid levels and changes in body fat distribution in treatment-naive, HIV-1-Infected adults treated with rilpivirine or Efavirenz for 56 weeks in the ECHO and THRIVE trials |                                                    |
|     |      | Mechanisms of zidovudine-induced mitochondrial toxicity and myopathy     | Myopathy<sup>181</sup>                            |
| 5.  | Didanosine (ddI) | Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access: | Lactic acidosis, hepatic toxicity, pancreaticitis and peripheral neuropath, Mitochondrial dysfunction<sup>157</sup> |
|     |      | Incidence of pancreatitis in HIV-infected patients: comment on findings in EuroSIDA cohort | Pancreatitis<sup>182</sup>                        |
|     |      | Didanosine. An update on its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV disease | [183]                                               |
| 6.  | Stavudine (d4 T) | Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access | Hyperlipidemia, hyperglycemia, insulin resistance, diabetes mellitus, osteopenia, osteoporosis and osteonecrosis, lactic acidosis, hepatic toxicity, pancreaticitis and peripheral neuropath, Mitochondrial dysfunction<sup>157</sup> |
|     |      | HIV drug stavudine (Zerit, d4 T) and symptoms mimicking Guillain–Barré syndrome | Neuromuscular weakness<sup>184</sup>               |

(Continues)
TABLE 1 (Continued)

| 7. | Stavudine and didanosine combination | Neurological and psychiatric adverse effects of antiretroviral drugs | Peripheral neuropathy\textsuperscript{185} |
|    |                                   | The risk of developing peripheral neuropathy induced by nucleoside reverse transcriptase inhibitors decreases over time: evidence from the Delta trial | [186] |
|    |                                   | Improvement of dyslipidemia in patients switching from stavudine to tenofovir: preliminary results | Hyperlipidemia\textsuperscript{179} |
|    |                                   | Lipid levels and changes in body fat distribution in treatment-naive, HIV-1-Infected adults treated with rilpivirine or Efavirenz for 96 weeks in the ECHO and THRIVE trials | [180] |
| 8. | Abacavir                          | Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients | Myocardial infarction\textsuperscript{187} |
|    |                                   | Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: a population-based nationwide cohort study | [188] |
|    |                                   | Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons | [189] |
| 9. | Tenofovir disoproxil fumarate     | Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 update by the HIV Medicine Association of the Infectious Diseases Society of America | Nephrotoxicity\textsuperscript{163} |
|    | (Tenofovir DF)                    | Drug-induced acute interstitial nephritis mimicking acute tubular necrosis after initiation of tenofovir-containing antiretroviral therapy in patient with HIV-1 infection | Interstitial nephritis\textsuperscript{190} |
| 10. | Tenofovir alafenamide             | Tenofovir alafenamide versus tenofovir disoproxil fumarate, Page 23/60 coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomized, double-blind, phase 3, non-inferiority trials | Increase in lipid parameters (total cholesterol and HDL)\textsuperscript{191} |
| 11. | Dolutegravir                      | Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection | Insomnia\textsuperscript{192} |
|    |                                   | Dolutegravir: a next-generation integrase inhibitor for treatment of HIV infection | Myopathy\textsuperscript{193} |
| 12. | Rilpivirine                       | Rilpivirine versus efavirenz-based single-tablet regimens in treatment-naive adults: week 96 efficacy and safety from a randomized phase 3b study | Neuropsychiatric side effects, depression and insomnia\textsuperscript{194} |
|    |                                   | Neurological and psychiatric tolerability of rilpivirine (TMC278) versus efavirenz in treatment-naive, HIV-1-infected patients at 48 weeks | [195] |
| 13. | Atazanavir                        | In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation | Hyperbilirubinemia\textsuperscript{196} |
|    |                                   | Urolithiasis in HIV-positive patients treated with atazanavir | Neprolithiasis\textsuperscript{197} |
|    |                                   | Complicated atazanavir-associated cholelithiasis: a report of 14 cases | Cholelithiasis\textsuperscript{198} |
| 14. | Indinavir                         | Crystalluria and urinary tract abnormalities associated with indinavir | Nephrotoxicity, kidney stone\textsuperscript{199} |
| 15. | Lopinavir-Ritonavir               | Lopinavir/ritonavir: a review of its use in the management of HIV infection | Alcohol in liquid formulation\textsuperscript{200} |
| 16. | Tipranavir/ritonavir              | Intracranial hemorrhage and liver-associated deaths associated with tipranavir/ritonavir: review of cases from the FDA’s Adverse Event Reporting System | Intracranial hemorrhage, Hepatotoxicity\textsuperscript{201} |
| 17. | Protease Inhibitors               | HIV protease inhibitors activate the unfolded protein response in macrophages: implication for atherosclerosis and cardiovascular disease | Insulin resistance, Atherosclerosis, cardiovascular disease\textsuperscript{202} |
| 18. | Maraviroc                        | Hepatic safety of maraviroc in patients with HIV-1 and hepatitis C and/or B virus: 144-week results from a randomized, placebo-controlled trial | Hepatotoxicity\textsuperscript{203} |

This table delineates the toxicity profile of non-nano antiretroviral drugs and the recent studies on non-nano antiretroviral drugs with their various toxic effects on organ profiles.
TABLE 2 Toxicity profile of antiretroviral drugs loaded nanoparticles

| S/N | ARVDS loaded NPS | Studies | Toxicities/activities |
|-----|------------------|---------|-----------------------|
| 1.  | ARV loaded lactoferrin nanoparticles | Evaluation of the reproductive toxicity of antiretroviral drug loaded lactoferrin nanoparticles | Significant decrease in litter size^{209} |
| 2.  | Dapivirine-loaded nanoparticles | Polymeric nanoparticles affect the intracellular delivery, antiretroviral activity and cytotoxicity of the microbicide drug candidate dapivirine | Improved antiviral activity compared to free drug^{85} |
| 3.  | Poly-(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) containing ritonavir (RTV), lopinavir (LPV), and efavirenz (EFV) | Combination antiretroviral drugs in PLGA nanoparticle for HIV-1. | No significantly cytotoxicity^{21} |
| 4.  | Poly(alkylcyanoacrylate) saquinavir loaded nanoparticles | Formulation and cytotoxicity of combined cyclodextrin poly(alkylcyanoacrylate) nanoparticles on Caco-2 cells monolayers intended for oral administration of saquinavir | Decreased cytotoxicity^{210} |
| 5.  | Poly(lactic-co-glycolic acid) zidovudine-lamivudine nanoparticles | Formulation and in vitro evaluation of zidovudine-lamivudine nanoparticles | Acute toxicity to animal cells was not detected^{211} |
| 6.  | Poly-(dl-lactide-coglycolic acid; PLGA) containing efavirenz (EFV) and boosted lopinavir (lopinavir/ritonavir; LPV/r) | Polymeric nanoparticles containing combination antiretroviral drugs for HIV type 1 treatment | No cytotoxicity seen for 28 days of treatment^{207} |
| 7.  | ARV (zidovudine, lamivudine, nevirapine, and raltegravir)-loaded PMM-based nanoparticles | Antiretroviral Drugs-Loaded Nanoparticles Fabricated by Dispersion Polymerization with Potential for HIV/AIDS Treatment | CEM cells and PBMCs culture toxicity at higher concentration (CC_{50} = 42 Mm^{212}) |
| 8.  | Raltegravir gold nanoparticle and penetration into the brain in vivo without toxicity | Gold nanoparticles to improve HIV drug delivery | No neurotoxicity found^{213} |

This table depicts the different nanomaterials, organ toxicities, and the recent studies of nanomaterials with their various toxic effects on organ profiles.

6 | INTERPLAY BETWEEN NANOMEDICINE: ACHIEVING DRUG EFFICACY, ADEQUATE BIOAVAILABILITY, AND BALANCING TOXICITY

Nanomedicine plays a crucial role in achieving biological barrier penetration and drug delivery efficacy while balancing toxicity owing to their physicochemical properties. The contemporary method of loading antiretroviral drugs with NPs has previously been reported to reduce adverse side effects of antiretroviral drugs as well as required dosage, which lessens the drug resistance and ensures drug potency.^{52}

Premature release of the drug has been described as an impendiment to intracellular and systemic diseases and infections.^{225} Moreover, steady and sustained drug delivery has been stated as an essential feature for retaining adequate concentrations of drugs within the beneficial range;^{226} which alleviates the likelihood of drug resistance.

Nanoparticles have been viewed as a tool to achieve increased drug efficacy with decreased potential toxicities owing to their ability to be kept in the body for a more extended period than traditional modalities,^{227} which aids steady and sustained delivery. It is, therefore, necessary to consider ways to increase drug efficacy while decreasing potential toxicities and the tendency of drug resistance. The study by Cauchetier et al^{228} described directing the nanoformulation to the specific site, thereby boosting drug efficacy.

Several mechanisms by which NPs lessen the toxicity of drugs have been reported. Nanoparticles can work as a substitute for the harmful solubilizing medium when administering hydrophobic agents.^{229,231} Additionally, enhanced permeability and retention (EPR) ability has been described as another mechanism by which NPs reduce the toxicity of drugs.^{232,234} Previous studies have also documented the ability of NPs to enhance absorption, distribution, metabolism, and elimination of drugs by reducing the toxicity of drugs that have build-up at the site of action. Moreover, boosting the curative effect of drugs by accelerating intracellular delivery and sustenance of retention period both in the systematic circulation and inside the cell are also recorded as other means by which NPs reduce the toxicity of drug.^{235,236}

In achieving drug efficacy and balancing toxicity, biological barriers are the determinant of the size-dependent biodistribution of NPs within tissue, organs, and surrounding fluid. A study indicated NPs penetrating ability to be a function of size. Hence, an increase in the size of NPs will bring about a decrease in barrier permeability.^{237} To achieve good penetration and avoid excessive accumulation that may
lead to toxicity, the size of NPs should be more than 10 nm,238,239 and 20 nm or less to achieve the most significant permeability or penetration.240-242 Activation of the complement system and accumulation of NPs in the spleen and liver resulted from the administration of NPs with a diameter of more than 200 nm238,243,245 have been documented.

Conversely, considering HIV infection, substantial accumulation of NPs in the macrophages, which also serves as a sanctuary region for HIV, may likely present a therapeutic advantage. More significant accumulation within the macrophages may worsen cell physiological activities.244 Nevertheless, enhancement of safety by reducing the dosage, adverse effects, and boosting biodistribution to the infected cells are pivotal to the invention of nanomedicine.52,244 A previous study revealed that NPs reduce the toxicity of primary hydrophobic therapeutic agents such as antiretroviral drugs by boosting their solubility and strengthening their stability, shielding them from non-specific regions.52

A recent study shows that antiretroviral drugs’ efficacy depends on the distribution and sustenance of adequate dosage at the

### TABLE 3 Nanomaterials and organ toxicities

| S/N | Nanomaterial | Study | Organ toxicity | References |
|-----|-------------|-------|----------------|------------|
| 1.  | Gold nanoparticles | Reversible cardiac hypertrophy induced by PEG-coated gold nanoparticles in mice | Chronic cardiac toxicity | 126 |
|     |             | Application of gold nanoparticles in biomedical and drug delivery | | |
|     |             | Cytotoxic effects of gold nanoparticles exposure employing in vitro animal cell culture system as part of nanobiosafety | Spleen, Lung | 214,215 |
| 2.  | Carbon nanoparticles (CNP) | A comparison of dispersing media for various engineered carbon nanoparticles | Largest CNP agglomerates in lung | 216 |
| 3.  | Zinc oxide (ZnO) nanoparticles (NPs) | Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle-exposed human immune cells | Cytotoxicity | 217 |
| 4.  | Silver nanoparticles | In vitro toxicity of nanoparticles in BRL3A rat liver cells | Cytotoxic effects on HepG2 cell line and primary liver cells of mice | 218 |
| 5.  | ZnO nanoparticles | Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats | Nephrotoxicity (mitochondria and cell membrane impairment in rat kidney) | 219 |
| 6.  | Titania (TiO2) nanoparticles | Cytotoxic and genotoxic impact of TiO2 nanoparticles on A549 cells | Cytotoxic and genotoxic impact on a cell line representative of human lung | 220 |
| 7.  | Mn3O4 nanoparticle | Toxic effects of Mn3O4 nanoparticles on rat testis and sex hormone | Reduction in testicular cytology | 154 |
| 8.  | Titanium oxide nanoparticles | Unraveling the neurotoxicity of titanium dioxide nanoparticles: Focusing on molecular mechanisms | Neurotoxicity | 155 |
| 9.  | Silica nanoparticles | Silica nanoparticles induce neurodegeneration-like changes in behavior, neuropathology, and affect synapse through mapk activation | Neurodegeneration disorders | 221 |
| 10. | Polyethylene glycol (PEG) | Assessment of PEG on polymeric particles surface, a key step in drug carrier translation | Immuno-toxicity | 222 |
|     |          | Subchronic toxicity and immunotoxicity of MeO-PEG-poly (D, L-lactic-co-glycolic acid)-PEG-OMe triblock copolymer nanoparticles delivered intravenously into rats | | 223 |
| 11. | Cerium oxide nanoparticles | SF-1 mediates reproductive toxicity induced by Cerium oxide nanoparticles in male mice | Testis impairment and sperm DNA damage | 152 |
| 12. | Anatase TiO2 nanoparticles (NPs) | Toxic effects of anatase titanium dioxide nanoparticles on spermatogenesis and testicles in male mice | Sperm malformation and Spherospermia | 153 |
| 13. | Iron oxide nanoparticles (FeNP) | Effects of iron oxide nanoparticles on mouse sperm parameters and testicular tissue | Reduction in testicular interstitial tissue volume, Reduction in the sperm parameters | 224 |

This table describes the different nanomaterials, organ toxicities, and the recent studies on nanomaterials with their various toxic effects on organ profiles.
specific site for the recommended period. Several studies have reported that loading antiretroviral drugs with NPs appears to be a breakthrough in ensuring drug efficacy at reduced doses. Recently, a finding delineated the great translational prospects of antiretroviral drug-loaded NPs to aid drug compliance and reduce viral resistance based on its sustained delivery system and targeted efficacy with little toxicity.

Interestingly, owing to emerging shreds of evidence and their physicochemical characteristics, antiretroviral drugs can be separately loaded with particles of nano-size to effectively combat HIV infection. Recent findings have accredited 50-double curtailment in indices of antiretroviral drug-loaded NPs, as well as a 50-fold improvement in antiviral effects when compared to free antiretroviral drugs, which establishes the effectiveness and activities of antiretroviral drug-loaded NPs compared to free antiretroviral drugs.

However, with the advent of NPs coupled with antiretroviral drugs, a few studies have documented their toxicity and adverse effects, suggesting that NPs could not completely eradicate the issue of toxicity. This suggestion indicates that structural architecture and morphometric assessment of specific organs or tissues should be considered in formulating the NPs.

Therefore, time of release, duration in the body, route of administration, biological barriers, drug transporters, and delivery methods all play a crucial role in achieving drug efficacy and adequate bioavailability.

7 | STEREOLOGICAL CONSIDERATION

Design-based stereology has been reported to be a useful tool because of its application to different organs. More so, it has been described as an appropriate tool to assess the precise morphological and morphometric parameters. Design-based stereology can be utilized to extrapolate two-dimensional objects to three-dimensional objects concerning advanced stochastic and statistical information. Furthermore, a three-dimensional profile has been regarded as an integral feature of stereology and quantification devices. Hence, incorporating stereological techniques with 3D radiological procedures such as volume electron microscopy, small computed tomography, and confocal microscopy would analyze the broad sample size and give a perfect resolution.

Stereology has been widely applied in morphological and morphometric research. It is a combination of quantitative and comparative approaches that utilize lines, points, numbers, length, area, volume, and planes to evaluate three-dimensional indices.

This method has been widely employed in neuro research, quantifying the microarchitecture of the kidney. Stereology has been employed to quantify the liver macrophages and hepatocytes. Also to assess the human lung pathologies as well as testicular morphological and morphometric parameters.

Previously, issues regarding penetration of the BTB and distribution of antiretroviral drugs to the viral sanctuary sites and the effect of the antiretroviral drugs on testicular morphology have received significant attention. A recent study has demonstrated the adverse effects of HAART on reproductive parameters employing qualitative histopathological methods and morphometric analyses and revealed that HAART causes detrimental histopathological changes in the testes leading to tubular atrophy with altered morphometric parameters.

However, very few stereological approaches have been recorded in assessing the adverse effects of antiretroviral drugs on reproductive indices. A recent review suggested that the stereological method is applicable in evaluating changes in testicular morphological parameters, volume estimation, biological reference spaces, and resulting damage on endocrine organs from the way they appear in two dimensions to three dimensions following an altered distribution of highly active antiretroviral therapy.

Testis presents an additional biological barrier that exists between the seminiferous tubules and vascular compartment, consequently favoring the tenacity of viral replication. There have also been reports of a decrease in antiretroviral drugs’ penetration through the BTB attributed to both the breast cancer resistance protein and efflux transporters P-glycoprotein. The application of NPs in drug delivery has offered new hope in treating HIV infections by enhancing antiretroviral drugs’ penetration through the BTB and improving therapeutic efficacy.

Over the years, pathologists have depended on the two-dimensional method to assess cell profile and cell numbers, but recently, research has proven that this method seems biased, assumption-based, and insensitive. Likewise, literature has reported limited sensitivity in detecting cell numbers based on qualitative analysis. Furthermore, quantitative data derived from an interpretation of the two-dimensional morphometric analysis method are usually assumption-based, inaccurate estimations. They are not the true reflection of the sample size and numbers. This fact is based on the literature that revealed that the resulting profiles are one dimension less than the actual when different objects of one, two, or three dimensions are subjected to a two-dimensional section plane. This finding implies that the two-dimension surface would produce a one-dimensional profile, and a three-dimension profile produces a two-dimensional shape.

In the same vein, for precise changes in cell number and structure to be appreciated and well defined, a sensitive qualitative evaluation such as a stereological method is required. Stereological methods provide an experimental and technically reasonable way of getting a concise and correct qualitative assessment of morphological changes in the tissue obtained from the histological sections. Besides, where other qualitative analyses discover changes in tissue morphology at 25%–40%, though depending on tissue type, the stereological method picks it up earlier.

Although wide attention has been given to applying the stereological method in quantifying testicular parameters, few studies have been done on the stereological quantification of testicular parameters of rats under antiretroviral drugs. To date, very few articles have documented the stereological approach in antiretroviral drug-loaded nanoparticles. In a recent study, a stereological
method was used to investigate the toxicity profile of Tenofovir and Tenofovir nanoparticles on the liver and the kidney of experimental rats. This finding shows accurate stereological assessment, as there were no significant changes in the kidney’s morphological parameters and that of control rats in both stereological approach, Renal function test, Liver function test, and cell count.266

The blood–testis barrier is unique. Aside from the tight junction (TJ) and gap junction (GJ) that are also found in other barriers, the BTB also contains the adherens junction (AJ), ectoplasmic specialization (ES), desmosome, hemidesmosome, and tubulobulbar complex (TBC)107,108 which could be considered in the formulation of drugs loaded with NPs. Therefore, it is imperative to consider employing a stereological approach in describing abnormalities of testicular morphology, quantitative estimation of antiretroviral drugs reaching seminiferous tubules, and toxicity evaluation of NPs loaded with antiretroviral drugs in the nanocarrier formulation of HAART (Figure 3).

8 | CONCLUSION AND FUTURE PERSPECTIVES

While early studies on the effects of antiretroviral drugs on sperm (and testicular tissue) were derived from rodent models, there are now emerging new data (also in top high impact journals) revealing the diverse impact of antiretroviral drugs on the testes in humans.267-269 Besides, there are now issues related to sperm defects269,270 and viral replication and drug resistance271,272 on the rise in HIV patients under antiretroviral treatment. These issues are partly attributed to the low drug concentration in the sanctuary sites with insufficient delivery to confer a competitive advantage in combating viral replication and achieving therapeutic efficacy.273 The literature has also reported adverse and toxic effects of these antiretroviral drugs or HAART because the entire body is exposed to multiple drugs at high doses. Therefore, it is necessary to explore means of achieving targeted delivery to anatomical sanctuary sites (including the testes) nanotechnology. Nevertheless, reducing the viral load to improve the quality of life of HIV-infected patients has been the cornerstone in the management of HIV infection. Nano-delivery systems have become the appropriate means for efficient delivery of drugs to these sanctuary sites to combat viral replication, rebound, and adverse effects of antiretroviral drugs on testicular morphology.273

Therefore, nanomedicine has given a temporary breakthrough in this regard. Nanoparticles are now relevant in drug delivery because of their ability to penetrate the so-called “anatomical sanctuary sites” such as the brain and the testis, which have previously been reported to be challenging to penetrate, especially for antiretroviral drugs or HAART. This advancement in nanomedicine enables antiretroviral drug-loaded nanoparticles to deliver a substantial quality of antiretroviral drugs to these sanctuary sites. However, some researchers have documented different adverse effects and toxicities of NPs on organs of the body, ranging from the testis, brain, kidney, liver, spleen, lung, and on various biochemical parameters. Still, little information is available on the toxicological evaluation and mechanism of toxicity of antiretroviral drug-loaded nanoparticles. Additionally, it is becoming difficult to differentiate HIV infection complications, antiretroviral drug adverse effects, and nanoparticle toxicities. In light of this, future research on the morphology of the specific organ of study in formulating the antiretroviral drug-loaded nanoparticles to reduce the toxicity profile while achieving drug delivery efficacy should be conducted. More studies are also needed to substantiate the causes of toxicity in antiretroviral drug-loaded nanoparticles and fully understand their mechanism of toxicity. Imperatively, an animal experiment should be set up to evaluate the toxicity of testicular morphology and BTB in the nano delivery of antiretroviral drugs using a stereological approach.

FIGURE 3 Stereological method on assessment of toxicity profile of testicular morphology in nano-delivery of highly active antiretroviral therapy. This figure describes the stereological evaluation of the testicular tissue when a nano-delivery system is employed to deliver antiretroviral drugs through blood-testis barrier. (A) Loading of antiretroviral drugs with nanoparticles. (B) Delivery of nanoparticle-loaded antiretroviral drugs through blood-testis barrier to reach testis. (C) Stereological approach in assessment of toxicity of testicular morphology.
ACKNOWLEDGMENT
The authors appreciate Professor Roshila Moodley and Dr. Oseni Shina for their editing services.

DISCLOSURE
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Naidu ECS, Olojede SO, Azu OO, Lawal SK, and Rennie CO conducted experiments, performed data analysis, participated in research design, and wrote or contributed to the writing of the manuscript.

DATA AVAILABILITY STATEMENT
Not applicable.

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