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

Abhishek Singh Thakur[1], Snehal Namdeo[2], Virendra Vaishnav[3], Dorendra Deshmukh[4], Bholenath Sahu[4] , Jaya Mishra[5] , Hari Prasad Sonwani**

[1]Apollo College of Pharmacy, Durg 491001(Chhattisgarh),India
[2]APJ Institution kanker, Chhattisgarh
[3]Faculty of Pharmacy, Bharti Vishwavidyalaya , durg Chhattisgarh
[4]School of Pharmacy, Shri Shankaracharya Professional University, Junwani Road, Bhilai, Durg, Chhattisgarh – 490020
[5]Riyansh College of Pharmacy, Village-Taparkela-Khurd, Lakhanpur, Dist:- Surguja (C.G.),India
Corresponding author : Hari Prasad Sonwani

Abstract :

One drug delivery method that has a lot of promise for treating HIV infections is the conjugation of nanoparticles (NPs) with antiretroviral medications. Recent research has brought attention to the harmful effects of nanoparticles on testicular tissue and their influence on sperm shape, notwithstanding their potential. When using a nanoparticle drug delivery technology in highly active antiretroviral therapy (HAART), this review examines the function of stereological techniques in evaluating testicular morphology. Additionally, a crucial component of this review is the pharmacokinetics and penetration of NPs with respect to the blood–testis barrier and testicular tissue. Furthermore, different kinds of NPs used to administer antiretroviral medications in clinical and biological research were covered in detail. Additionally, factors for reducing the toxicity of nanoparticles to pharmaceuticals, guaranteeing improved nanoparticle permeability, increasing therapeutic efficacy, guaranteeing sufficient bioavailability, and formulation and manufacture of HAART-NPs are extensively discussed.

Keywords : nanoparticles (NPs), HAART, antiretroviral therapy, testicular, blood–testis barrier

Important Remark

When using a nanoparticle to administer antiretroviral medications, this minireview shows that the drug's route of administration, dosage, length of therapy, and drug transporters are all crucial in reducing toxicity and optimizing drug efficacy and bioavailability. Stereological measurement of the target organs' morphology and cell and cell type can be used to minimize drug toxicity while maintaining optimum pharmacological efficacy.

Introduction :

It has been proposed that the testis contains a protective "sanctuary site" that restricts drug concentration or sequestration.1–3 The testis, a crucial reproductive organ that is compartmentalized within the scrotum, is a symbol of this sanctuary site. The tunica vaginalis, tunica albuginea, and tunica vasculosa are the thick layers of connective tissue capsules that enclose it from the outermost to the interior.The testis is divided into distinct lobules by four septa

from the tunica albuginea. roughly 60% of the testis' entire volume is made up of seminiferous tubules, which are roughly 200 µm in diameter and 600 m long overall. These tubules are found inside each of these lobules.5, 6 This protected area of the seminiferous tubules7, which is divided by the blood–testis barrier (BTB), is where spermatogenesis takes place. This division of labor offers a barrier between the vascular environment, which offers a supportive environment for spermatogenesis, and the region of spermatogenic cells.

One organ that may harbor the human immunodeficiency virus 1 (HIV-1) is the testis.9, 10 A number of studies have documented negative effects of antiretroviral medications (ARVDs) on reproductive parameters, indicating that the overall effects of highly active antiretroviral therapy on the testis have not been adequately taken into account. Deleterious effects of highly active antiretroviral therapy on sperm motility continue to add to the debate.11. However, research has shown that HIV-1 patients receiving highly active antiretroviral therapy (HAART) do not have any changes in their semen parameters. Twelve However, alterations in HIV-1 patients' semen characteristics while on HAART have been proposed,13 with probable HAART's detrimental impact on the reproductive system.14 This includes a notable decrease in overall sperm motility in the animal groups receiving lamivudine, nevirapine, and zidovudine as part of HAART.15, 16 All of these studies have demonstrated that HAART enters the seminiferous tubules, albeit in smaller amounts because to the blood–testis barrier (BTB). However, in many individuals, HAART has been successful in reducing viral load, viral replication, and Cell of Differentiation 4 (CD4) counts to undetectable levels.17

The uptake of ARVDs into the testis is considerably decreased by the BTB that separates the vascular compartment of the testis from the seminiferous tubules. This decrease is a result of the efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) working together to prevent and limit the entrance of ARVDs.17-20 Nevertheless, infusing ARVDs with nanoparticles can help get around the problem of BTB penetration. According to a few research, ARVDs that are nano formulated have permeated the BTB. It has been demonstrated that poly (lactic-co-glycolic acid) (PLGA) nanoparticles can accumulate lopinavir, ritonavir, and efavirenz in peripheral blood mononuclear cells in the testis of mice for 28 days without causing cytotoxicity.21

NANOPARTICLE PENETRATION IN HAART FROM BENCH TO CLINIC

The prevalence of infectious and noncommunicable diseases remains high despite advances in medical detection and treatment technology. To reduce mortality, point-of-care diagnostic techniques that are easy to use, affordable, quick, and sensitive are required, as are medication regimens with fewer side effects and toxicity.22 As the horizon expands and business interest in nanomedicine grows, nanoparticles with special qualities are being added to a variety of products. There are already over 500 consumer items on the market that claim to include nanoparticles, and more are constantly being discovered.23, 24 Human exposure to nanoparticles tends to rise as a result of this uncontrolled use. It is necessary to validate the characterization procedures, predicted toxicities, and hazard capabilities of nanomaterials and nanodevices.25 Nanomaterials have been utilized in medicine and have been appealing for technological advancement in the fundamental sciences The creation of nanotechnology and the application of ultramicroscopic particles that are

invisible to the naked eye are not recent developments26, which is how nanomedicine came to be. As tiny technology platforms are used in biomedicine to address medical issues, this field is embracing an expanding number of them.27 Over the next ten years, it has the power to totally influence, guide, and transform medical treatments.

Classes of nanoparticles

HIV particles are comparable in size to nanoparticles (NPs), which are materials with a molecular weight of about 100 nm.28 Inhalation is one among the easier ways that NPs can enter the bloodstream and organs, though there are other ways as well. Although many NPs are safe and have positive benefits, certain NPs have also been shown to have toxicities.29 Based on their characteristics and wide range of uses, nanoparticles are divided into several groups. These consist of lipid-based nanoparticles (LBNPs), metal nanoparticles (MNPs), semiconductor nanoparticles (SCNPs), ceramic-based nanoparticles (CBNPs), polymeric nanoparticles (PNPs), and carbon-based nanoparticles (CNPs) Due to their optical and electrical properties, metal nanoparticles have drawn a lot of interest for use in clinical and medical settings.30 The properties that have made silver, gold, zinc, cadmium, platinum, copper, and iron popular for use in the synthesis of nanoparticles are their huge surface area to volume ratio, enormous area energies, quantum detention ability, and the ability to absorb and store a large number of electrons.31 32 MNPs made of copper, gold, and silver are being developed as drug carriers for application in bioimaging, diagnostics, and treatment because of their physicochemical characteristics.

Derivatives of elements, compounds, or a mixture of two or more elements that fall between metals and nonmetals in groups IV and VI of the periodic table are known as semiconductor nanoparticles. Nanoparticles of semiconductors, including silicon (SiNPs), germanium (GeNPs), In the fields of electrical, optical, electronics, and fiber networks, tin (SnNPs), selenium (SeNPs), tellurium (TeNPs), zinc oxide (ZnO), zinc sulfide (ZnS), cadmium sulfide (CdS), cadmium selenide (CdSe), and gallium nitride (GaN) are employed.33

The inorganic nonmetal solids that make up ceramic-based nanoparticles can be amorphous, porous, or polycrystalline.34 These nanoparticles are employed in dye photodegradation, photocatalysis, and medical imaging.35 Additionally, CBNPs like aluminum oxide (Al2O3) and titanium dioxide (TiO2) have been used extensively in the production of nano delivery systems36, whereas silica, albumin, and iron oxide are used in drug delivery systems.37, 38

The most often used CNPs include graphite, graphene, carbon nanotubes, nanodiamonds, and Buckminsterfullerene (C60).39 A few of these CNPs have the ability to create carbon nanotubes, such as graphene41, which have been utilized in medicinal as well as cellular labeling agents or drug delivery systems.40–42 There have been reports of Buckminsterfullerene being used therapeutically as an anti-HIV drug.43

Organic-based NPs having solid mass encased in a particle are known as polymeric nanoparticles (PNPs).44 Large molecular architectures, glass transition, long-chain participation, and crystallization performance are characteristics that set polymers apart.45 Drug delivery methods

employ carboxy-terminated poly(D,L-lactic-co-glycolide)-block-poly (ethylene glycol) (PLGA-b-PEG-COOH) and poly(D,L-lactic-co-glycolide)/montmorillonite (PLGA/MMT) PNPs.46 and 47

Functional lipids found in lipid-based nanoparticles (LBNPs) enable them to eventually be tolerated and broken down into a harmless precipitate. Because of their efficacy and safety in drug delivery systems, LBNPs such ethosomes, lipid nanoemulsions (LNE), liposomes, transfersomes, solid lipid nanoparticles (SLNs), and niosomes have drawn a lot of attention during the past ten years.48, 49 Additionally, biocompatibility, ease of preparation, and good thermal stability The benefits of LBNPs include robust loading capacity, biodegradability, cost-effectiveness, and large-scale preparation.50 and 51

Application of nanoparticles in medicine

By improving the effectiveness and delivery of highly active antiretroviral medications to the targeted organs, the developing field of nanotechnology has the potential to transform the current HIV treatment approach.52–56 The use of different NPs that can cross the blood-testis barrier has been credited with this new direction.57 The intracellular drug delivery system employing NPs has been described in a number of investigations. The key mechanisms of action include receptor-mediated phagocytosis of nanocarriers, passive diffusion of free pharmaceuticals, nonspecific phagocytosis of nanocarriers, and the pinocytosis process of nanocarrier uptake.

Certain intracellular drug delivery systems may use multiple mechanisms in conjunction. When the NP is released within the lysosome, the medicine may be broken down, making the treatment useless. Nonetheless, successful treatment is possible when medications are discharged into the cytosol.58–60 Immunodeficiency virus-1 infection is indulged by testis CD68+ macrophages, which promote viral replication without interfering with testosterone release.61

According to the earlier research, the evolving interplay between metabolism, drug efflux, and influx determines how well antiretroviral medications can enter anatomical compartments, anatomical sanctuary areas, and viral reservoir sites. These have been linked to the virus's susceptibility to antiviral medications, viral persistence, and ineffective viral suppression.62 There is growing evidence that ATP-binding cassette transporters (ABC) are one of the key elements that prevent medications from entering the testes. Additionally, research has shown that the testes may delay the entry of antiretroviral medications and serve as a harbor for HIV-1, which could result in ongoing HIV-1 infections and the resulting resistance to medication.9

In order to get a successful outcome in the management of HIV infections, a prior study found that striking a balance between the antiretroviral medications' efficacy, safety, permission, and administration is crucial.63. Unfortunately, due to documented toxicities, adverse effects, and side effects, these antiretroviral medications—particularly the nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs)—have come under fire.Two Nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI), in

particular, have been implicated in a number of studies as being associated with insulin resistance and the potential development of diabetes mellitus in individuals living with HIV.64.-66. Similarly, lipodystrophy syndrome and gastrointestinal disorders have been reported.67. Cardiovascular disease is another serious side effect that has been reported significant hyperbilirubinemia and liver toxicity 68, 69.70 These side effects have caused individuals to stop taking their medications, alter their therapeutic approaches, and stop receiving therapy altogether.71

Tissue-specific drug-targeted techniques have been shown to increase the drug's efficacy at low dosages while lowering side effects by regulating the drug's biodistribution in nonspecific tissues.72–74 The main objective in managing HIV infection has been defined as reducing the toxicity of antiretroviral medications without compromising the therapeutic concentration and suppressing the viral load to an undetectable level. The effectiveness of NPs laden with antiretroviral drugs has drawn a lot of attention in this area. The use of nanotechnology in HIV therapy has been highlighted by Ochekpe et al.75 as a key component of drug delivery systems that tackles the bioavailability problem tissue distribution, imbalance in drug levels, and reducing the harmful effects of typical antiretroviral medications.75 Similarly, NPs laden with antiretroviral medications have been identified as a drug delivery method that guarantees a reduction in antiretroviral medication adverse effects.76

Drugs incorporated into nanoparticles have a bright future in nanotherapeutics due to their capacity to cross biological membranes.52 Nanomaterials have drawn a lot of attention, and their use in medication delivery systems to lessen toxicities and side effects has grown. Synthesized NPs have been given priority in order to accomplish a variety of applications in the field of nanomedicine. However, due to Royal Society and Royal Academy of Engineering restrictions, not all nanoparticles can be used in this way.77 These NPs have several qualities that make them extremely relevant, including their significant surface to mass ratio, which turned out to be greater than that of other particles, their ability to absorb and pick up other molecules, and their quantum properties. The main function of NPs' greater surface is to adsorb, bind, and gather up other materials like proteins and medications.78

The toxicity of NPs laden with antiretroviral drugs is also influenced by the production process. There are two methods for producing NPs: the top-down method, which involves reducing the production of bulk products, and the bottom-up method, which involves combining ingredients to create bigger particles.79 There has already been discussion of the green, chemical, and physical synthesis of NPs. More attention has been paid to green synthesis techniques than to physical and chemical processes due to their innate capacity for reduction and stabilization. As a result, the biosynthesis of NPs using microorganisms is gaining greater attention these days.80

While green synthesis techniques that use biological methods, irradiation methods, polysaccharides, and blended-valence polyoxometalates should be used, the usage of chemicals, which are typically associated with risks, should be eliminated in order to preserve ecologically safe operations. Furthermore, green synthesis has several advantages over methods that use chemicals that pose environmental risks, so it should be used. During the green manufacturing of

NPs, extra attention must be paid to selecting a solvent and hazardous-free stabilizing and reducing agents.81 and 82 According to a prior study, the synthesis technique of NPs and materials determines their constant release and efficacious therapeutic drug delivery.83

Thus far, just A few research have looked into how well HAART or NPs loaded with antiretroviral drugs can pass through the blood-testis barrier. Prior studies have documented the dispersion and accumulation of nano-coupled antiretroviral medications in mouse testes, including lopinavir, ritonavir, and polylactic-co-glycolic acid nanoparticles loaded with efavirenz.21 This finding suggests that NPs should be used to deliver antiretroviral medications to the male reproductive system.

Although great efforts have been made to develop HAART NPs that are effective against a variety of HIV-1 strains, reports of toxicity leading to DNA damage have surfaced.52 A small number of nanoparticles (NPs), including polymeric, liposome, and silver/gold, have been shown to improve the administration of antiretroviral medications to prevent or treat HIV infection.84 Ritonavir, lopinavir, and efavirenz in combination with dipivefrine and PLGA NPs21 Among various nano-formulated HAARTs, poly(~□-caprolactone) NPs85 has been reported to be at the preclinical stage.

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

The physiochemical properties of NPs play a major role in their pharmacokinetics (absorption, distribution, metabolism, and excretion).86 Both solid and liquid nanoparticles can pass through the tract's barriers and related physical, biological, and chemical processes, changing and transforming their pharmacological and toxicological characteristics in the process.87 Particle size is a crucial consideration; the smaller the particle size, the more potent the disease-curing effects. Therefore, when compared to medications of the same size, NPs produced drugs have superior metabolism, more widespread dispersion, correct absorption, stronger penetration, and greater bioavailability.88

There are several routes to give nanoparticles, including injectable, pulmonary, nasal, oral, and percutaneous.89 After being administered, NPs enter the bloodstream and are eliminated through feces or other channels.90 The epithelium and mucosal lining The gastrointestinal tract's tissue has been found to be the main obstacle to the absorption of medications made with nanotechnology. According to earlier research, intestinal enterocytes are the route by which NPs are absorbed.91 Furthermore, NPs in the 50–200 mm range are absorbed at the Peyer's patches on the wall of the small intestine.92

Different forms of NP absorption have been reported in a number of animal investigations, including through the epidermis, through lymph nodes and the lymphatic system, and through the nasal cavity, which is an excellent route for crossing the BTB95.

Additionally, it has been stated that the inhalation method, which involves NPs being absorbed by alveoli, is among the best methods due to the alveoli's increased surface area, which makes it easy for NPs to enter the lymphatic and blood circulation systems.94, 95 Additionally, different injectable techniques of NPs should be used while taking medication response and bioavailability into account.

The capacity to identify the most likely mode of action of NPs is a major advantage of their biodistribution.97 Following absorption, the composition, size, morphology, surface charge, and coating effects all affect the effective NP dispersion. According to their composition, mesoporous silica nanoparticles have been shown to have a higher affinity for the lungs than polymeric NPs have for the liver.97 In terms of size, for NPs to avoid the Hepatocytes in the liver must be smaller. This is justified by the shorter blood circulation time brought on by the liver and spleen absorbing larger particles.98, 99

NPs' biodistribution is mostly enhanced when they are coated with starch-like substances like dextran, polyethylene, and other coating materials.One hundred While metal NPs like silica, silver, iron oxide, and gold have complex metabolisms, the breakdown products of biodegradable NPs are simply digested. For example, according to a prior study, a quantum dot NP stayed in the body for two years.94 Moreover, iron oxide metabolism has been found to occur in astrocytes, which are supporting cells in the brain.102

There are various ways to get rid of NPs and medications, however the Renal excretion, a multiplex technique including tubular secretion and glomerular filtration, is the main process.103 Elimination through urine or feces is an additional technique.104 It's interesting to note that drug transporters at the BTB junctions have been significantly linked to drug pharmacokinetics. According to a recent study, the number of medications and chemical agents that reach the testis in both healthy and diseased states is determined by the drug transporters located in various BTB regions and junctions.105 and 106 Because of other, specific barriers, the BTB is a special blood barrier in the body. The adherens junction (AJ) and ectoplasmic specialization are also present in the BTB in addition to the tight junction (TJ) and gap junction (GJ), which are also present in other barriers (ES), tubulobulbar complex (TBC), hemidesmosome, and desmosome.107 and 108

The BTB keeps seminiferous tubules' adluminal and basal compartments apart, allowing sperm synthesis to proceed unhindered in the apical compartment behind the BTB. According to a prior study, the BTB's immunomodulatory action involves blocking the production of chemicals or aberrant antibodies that could impede the development of sperm.109 The BTB offers a conducive and wholesome environment for the generation of sperm.110

The tight junctions between Sertoli-Sertoli cells, actin-formed adherens junctions, and a cytoskeleton-based junction—most notably the intermediate filament-forming desmosome junctions—make up the majority of this barrier. The BTB served as the boundary between the adluminal and luminal compartments of the three compartments in the substance of seminiferous tubules.

compartments at the base. According to reports, these compartments are crucial for the growth of sex cells and shield them from NPs, foreign objects, hormone imbalances, poisons, and infectious

diseases so that they can continue to reproduce.111

The testis' functional unit, the seminiferous tubules, is encircled by myoid cells, which are contractile cells that drive the mature sperm from secretion into the epididymis, where they mature. However, Sertoli cells, an epithelium covering the deepest portion of the seminiferous tubule that serves to anchor and feed essential nutrients during sperm production, are where the BTB begins.112-114

Both practically and theoretically, nanoparticles are unable to flow through the BTB. However, other research using animal models showed that some NP properties permit penetration across the BTB. Larger particles, however, cannot pass through 115 According to Wang et al.116, NPs were able to pass through the BTB.116 According to a related study that looked at silver nanoparticles, small-sized NPs have the ability to pass through the BTB but large-sized NPs cannot.117

The ability of NPs to enter the testis and change spermatogenesis has been the subject of conflicting studies. NPs were not detected in the testis in a microscopic investigation.118 According to a different study, NPs can enter the testis, 119 pass through the BTB, and change how sperm are produced.120 The nontoxic effects of NPs on spermatogenesis were reported in another investigation.121 Crucially, findings from earlier research showed that a tiny amount of NPs were reaching the testis's contents regardless of the Administration technique 115, 122-124 The traditional method of assessing cytotoxicity and the efficacy of NPs may be complicated by the distinct properties of both BTB and NPs.125

On the other hand, NP penetration through testicular tissue has advanced significantly. However, certain issues including NP toxicity126 and the kind and characteristics of NPs to be used have not yet been resolved with reference to using NP drug delivery systems in basic and clinical research. According to a prior study by Papageorgiou et al.127, the toxicity profile of nanoparticles is determined by their size, chemical composition, surface characteristics, and crystalline characteristics. Another problem that needs to be resolved when using NPs in drug delivery systems is the synthesis method. The method of biogenic bottom-up synthesis has been examined. considered a superior approach due to its feasibility and non-toxicity, as proven by current research.128 When employing NPs in drug delivery systems, the procedure for loading NPs with medications must also be considered. It takes careful research to address the penetration of NPs in each of the junctions that make up the BTB.

NANOPARTICLES/NANOFORMULATIONS USED IN CLINICAL RESEARCH

While many of these NPs are still awaiting approval, only a few number have received permission for clinical usage to date. The Food & Drug Administration (FDA) and the European Medical Agency (EMA) have approved a number of NPs used in cancer, iron replacement, bacterial, and fungal therapies. The key NPs utilized in clinical diagnosis and treatment fall into two groups: inorganic NPs, which include metal and metal oxide NPs, and organic NPs, which comprise liposomal, protein-based, and polymeric NPs.129

Preclinical studies have shown the inorganic NPs to be effective. Iron oxide nanoparticles have been created and authorized for use in imaging and the treatment of anemia.130, 131 Organic nanoparticles, like liposomes, have been a huge success and have also been transformed into antifungal medications, vaccinations, and anesthetics.1, 132

To guarantee the efficient treatment of cancer, nanoparticles have been used in conjunction with anti-cancer medications. Phase III clinical studies on metastatic breast cancer used doxorubicin, an anti-cancer medication coated with pegylated liposomal HCl (CAELYX/Doxil).71 Gold nanoparticles were able to transfer medications to telomerase in another clinical study on heart disease, which in turn changed the growth of cancer cells.133 Gold NPs loaded with Levosimendan (Simdax) and gold NPs with a size of 30 nm demonstrated remarkable cardioprotective results in rats with doxorubicin-induced heart failure, significantly outperforming rats treated with Levosimendan (Simdax) alone, according to recent experimental research on the treatment of heart disease.134

The first FDA-approved nanoformulations for clinical studies were liposomes.

Beginning in the middle of the 1990s, liposomal versions of doxorubicin and amphoterin B were approved.135 Liposomal irinotecan, also known as Onivyde, was recently authorized as a second-line treatment for pancreatic cancer that has spread. Additionally, Marqibo (liposomal vincristine) was recently licensed to treat respiratory distress syndrome, fungus infections, multiple sclerosis, and pancreatic cancer.37, 136 The development of nanomedicines is still significant since there is convincing evidence that liposomal formulations have improved in nanotechnology and become clinically stable.37

Because of their superior protein stability and extended half-life, polymer nanoformulations like coagulation factor IX (Rebinyn) and antihemophilic factor VIII (Adynovate) have also been studied and approved for the treatment of hemophilia.137 Oncaspar (pegaspagase) was recently approved to treat a number of illnesses, including multiple sclerosis, prostate cancer, hepatitis, and chronic gut. The protein Because of their superior stability, enhanced delivery to the tumor, targeted T-cell specificity, and lysosomal escape, nanoformulations like Abraxane (albumin-bound paclitaxel) and Ontak (denileukin diftitox) have recently been approved to treat cutaneous T-cell lymphoma, pancreatic cancer, and breast cancer.137 Many of the new nanoformulations that the FDA receives each year for clinical studies have been approved for use in clinical settings.137 According to Clinical Trials.gov, 56 clinical trial nanoformulations had been received or were inactive as of October 2017.

In the clinical trial stage, iron oxide nanodrugs like Venofer Ferleccit have been thoroughly investigated. They are an approved indication for iron replacement therapy by the FDA.37 Iron oxide nanoformulations, however, are still being employed in clinical settings as a contrast-enhancing reagent for magnetic resonance imaging trial phase.138 For the treatment of HIV/AIDS, a number of NPs have received clinical validation. After proving safe and tolerated in preclinical and phase I clinical trials, the DermaVir patch was used for HIV/AIDS immunotherapy and subsequently advanced to stage II trials.139 Phase I/II trials are underway for an L-lysine

dendrimer.Preclinical trials are underway for 64, 141, 142 Silver NPs, 143, 144 dendrimers, 145, 146 Gold NPs, 147, and PGLA NPs148. Botegravir and rilpivirine, the first long-acting combination of antiretroviral medications, have been authorized for the treatment of HIV.149 It has recently been discovered that the long-acting injectable cabotegravir and rilpivirine can be treated for HIV infection using nanoformulation. Its benefits, including lowering the quantity of pharmaceuticals, limiting toxicity linked to drugs, lowering negative drug effects, and simplicity of treatment.150 Another work produced a crystal of myristoylated cabotegravir prodrug, which was then made into nanoparticles. In mice, the produced nano myristoylated cabotegravir (NMCAB) has been shown to enhance viral clearance patterns and biodistribution.151

Regarding the toxicity or hazards of the majority of NPs utilized in clinical studies or nanoparticles created for commercial application, there is no universal agreement. The toxicological profile of these NPs has been extensively studied by biomedical researchers, however the findings have not been sufficiently compelling. The organ toxicities of NPs have been extensively reported in recent years. Testicular cytology was reduced, testis impairment, sperm DNA damage, sperm deformity, asthenospermia, and toxicity in mice after chronic oral administration of CeO2 NPs were all evaluated in a previous work.154 Chronic cardiac toxicity, 155 Nephrotoxicity, 126 and provides a summary of NPs' other organ toxicities.

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

Numerous literary works have described a range of antiretroviral medication toxicities, from minor to severe adverse effects on the body's major organs and systems. The World Health Organization has now disclosed that it is now more challenging to differentiate between the typical consequences of HIV infection and the negative effects of antiretroviral medications.156, 157 Research has revealed toxicities, side effects, and even clinical adverse events associated with HAART, despite its many positive advantages.16 Clinical side effects such lipodystrophy, gastrointestinal issues, and hyperglycemia are linked to HAART.158

Combining several antiretroviral medications (HIV-HAART) exposes the body to high levels of each drug, which might restrict the therapeutic benefit, cause toxicity, or cause severe side effects. Several negative organ consequences of antiretroviral medications various bodily systems; zidovudine, azidothymidine, tenofovir disproxil fumarate, efavirenz, lamivudine, and stavudine have all been implicated in the suppression of bone marrow, which would subsequently lead to thrombocytopenia and anemia.157 and 159 In line with other studies in Table 1, the prior investigation documented peripheral neuropathy, lactic acidosis, hyperlipidemia, and insulin resistance as side effects of stavudine, 160–162, There are conflicting results about actual sperm functional tests, despite the increasing understanding of how HAART affects male fertility.16 Although it's unclear if the change was brought on by drug-induced diabetes, Onanuga et al. (2018) noted a significant histological change of the seminiferous tubule in the experimental mice receiving HAART and diabetes.204 Antiretroviral medications (Table 1), antiretroviral medications combined with NPs (Table 2), and the toxicity of these nanomaterials on many organs (Table 3) have all been documented in other investigations. However, antiretroviral medications

(such Saquinavir)205, 206, and combinations of several antiretroviral medications have been delivered more effectively thanks to the NP drug delivery system.207 There have been claims that gold and silver have antiviral qualities, against a variety of HIV-1 strains, but it presented significant toxicity problems that led to cellular death and DNA damage.52 Research has demonstrated that NPs or nanocarriers laden with antiretroviral drugs may recognize HIV-infected cells, distribute drugs to particular locations in the body, and administer repeated therapeutic dosages, all of which increase the effectiveness of the medicine.59, 85

TABLE 1. Toxicity profile of non-nano antiretroviral drugs

s.no	ARDS	Studies	Effects
01	Nevirapine	Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents Safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection Limitations to treatment safety and efficacy: adverse effects of antiretroviral agents	Hepatic necrosis Hypersensitivity Renal dysfunction
02	Efavirenz	A randomized cross-over study to compare raltegravir and efavirenz 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 Acute Liver Toxicity due to Efavirenz/Emtricitabine/Tenofovir	Persistent and troubling neuropsychiatric symptoms Hepatotoxicity
03	Raltegravir	Severe rhabdomyolysis associated with raltegravir use	Skeletal muscle toxicity, Rhabdomyolysis, and Elevated serum creatine kinase (CK)
04	Zidovudine, or azidothymidine	Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access	Anemia, neutropenia and, more rarely, thrombocytopenia
05	Didanosine (ddI)	Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access:	Lactic acidosis, hepatic toxicity, pancreatitis and peripheral neuropath,

			Mitochondrial dysfunction
06	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.
07	Stavudine and didanosine combination	Neurological and psychiatric adverse effects of antiretroviral drugs	Peripheral neuropathy
08	Abacavir	Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients	Myocardial infarction
09	Tenofovir disoproxil fumarate (Tenofovir DF)	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
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)

TABLE 2. Toxicity profile of antiretroviral drugs loaded nanoparticles

s.no	**ARVDS loaded NPS**	**Studies**	**Toxicity**
01	ARV loaded lactoferrin nanoparticles	Evaluation of the reproductive toxicity of antiretroviral drug loaded lactoferrin nanoparticles	Significant decrease in litter size
02	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

03	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
04	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 cyctotoxicity
05	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
06	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

TABLE 3. Nanomaterials and organ toxicities

Nanomaterial	**Study**	**Effects/Organ toxicity**
Gold nanoparticles	Reversible cardiac hypertrophy induced by PEG-coated gold nanoparticles in mice	Chronic cardiac toxicity
Carbon nanoparticles (CNP)	A comparison of dispersing media for various engineered carbon nanoparticles	Largest CNP agglomerates in lung
Zinc oxide (ZnO) nanoparticles (NPs)	Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle-exposed human immune cells	Cytotoxicity
Silver nanoparticles	In vitro toxicity of nanoparticles in BRL3A rat liver cells	Cytotoxic effects on $HepG_2$ cell line and primary liver cells of mice
ZnO nanoparticles	Zinc oxide nanoparticles cause nephrotoxicity and	Nephrotoxicity (mitochondria and cell membrane impairment in rat kidney)

	kidney metabolism alterations in rats	
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
Mn_2O_3 nanoparticle	Toxic effects of Mn_2O_3 nanoparticles on rat testis and sex hormone	Reduction in testicular cytology
Titanium oxide nanoparticles	Unraveling the neurotoxicity of titanium dioxide nanoparticles: Focusing on molecular mechanisms	Neurotoxicity
Silica nanoparticles	Silica nanoparticles induce neurodegeneration-like changes in behavior, neuropathology, and affect synapse through mapk activation	Neurodegeneration disorders
Polyethylene glycol (PEG	Assessment of PEG on polymeric particles surface, a key step in drug carrier translation	Immunotoxicity
Cerium oxide nanoparticles	SF-1 mediates reproductive toxicity induced by Cerium oxide nanoparticles in male mice	Testis impairment and sperm DNA damage
Anatase TiO_2 nanoparticles (NPs)	Toxic effects of anatase titanium dioxide nanoparticles on spermatogenesis and testicles in male mice	Sperm malformation and Spherospermia
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

As seen in Table 2, toxicity has recently been documented in a few of the NPs loaded with antiretroviral drugs. Lactoferrin NPs supplied orally resulted in a considerable reduction in litter size, according to Madugulla et al.209. However, the same medicines administered vaginally did not significantly alter litter size or postnatal development. This finding may indicate that the route of delivery may affect the toxicity of medications loaded with NP. Additionally, Ogunwuyi et al.212 found that NPs loaded with antiretroviral drugs (Nevirapine, Raltegravir, Zidovudine, and

Lamivudine) are hazardous at higher doses yet effective in inhibiting HIV-1 infection in CEM T cells and PBMCs.21

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

Because of its physicochemical characteristics, nanomedicine is essential for attaining biological barrier penetration and drug delivery efficacy while balancing toxicity. It has previously been reported that the modern approach of loading antiretroviral medications with NPs reduces the negative side effects of antiretroviral medications as well as the necessary dosage, which lowers drug resistance and guarantees drug potency.52

Early medication release has been characterized as a barrier to infections and intracellular and systemic illnesses.225 Furthermore, it has been said that maintaining appropriate drug concentrations within the advantageous range requires consistent and continuous drug delivery,226 which reduces the risk of drug resistance.

Because of their capacity to boost therapeutic efficacy while lowering possible toxicities, nanoparticles have been seen as a tool to be retained in the body longer than with conventional modalities,227 which facilitates a consistent and prolonged delivery. Therefore, strategies to improve therapeutic efficacy while reducing possible toxicities and the likelihood of drug resistance must be taken into account. In order to increase therapeutic efficacy, the work by Cauchetier et al.228 explained how to target the nanoformulation to a particular location.

There are a number of documented ways that NPs reduce the toxicity of medications. Hydrophobic agents can be administered using nanoparticles in place of the hazardous solubilizing medium.229–231 Furthermore, it has been shown that NPs also lessen the toxicity of medications through improved permeability and retention (EPR) capabilities.232-234 Additionally, prior research has shown that NPs can improve the absorption, distribution, metabolism, and excretion of by lessening the toxicity of medications that accumulate at the site of action. Additional ways that NPs lessen drug toxicity include increasing the therapeutic benefit of medications by speeding up intracellular delivery and maintaining retention periods inside the cell as well as in the systemic circulation.235, 236

The size-dependent biodistribution of NPs inside tissue, organs, and surrounding fluid is determined by biological barriers, which are crucial for attaining medicinal efficacy and balancing toxicity. According to a study, the penetrating capacity of NPs varies with size. Therefore, the barrier permeability will decrease as the size of NPs increases.237 For optimal penetration and to prevent overabundance that could cause toxicity, NPs' size should be greater than 10 nm, 238 nm, 239 nm, and 20 nm, or less, in order to attain the highest level of permeability or penetration.240–242 When NPs larger than 200 nm in diameter were administered, the complement system was activated and NPs accumulated in the liver and spleen.There are 238, 243, and 245 recorded.

On the other hand, given HIV infection, a significant buildup of NPs in the macrophages—which

also act as an HIV sanctuary—may probably offer a therapeutic benefit. A larger buildup inside the macrophages could exacerbate the physiological functions of the cells.244 However, the development of nanomedicine depends on improving safety by lowering dosage, minimizing side effects, and increasing biodistribution to the sick cells.52, 244 According to a prior study, NPs lessen the toxicity of main By increasing their solubility and fortifying their stability, hydrophobic medicinal agents—like antiviral medications—are protected from nonspecific areas.52

According to a recent study, antiretroviral medications' effectiveness is dependent on their distribution and maintenance of an appropriate quantity at the designated location for the suggested amount of time.208 According to a number of research, loading antiretroviral medications with NPs seems to be a novel way to guarantee medication efficacy at lower dosages. Based on its sustained delivery mechanism and targeted efficacy with minimal toxicity, a recent study outlined the significant translational prospects of antiretroviral medication-loaded NPs to help drug compliance and diminish viral resistance.246

Interestingly, antiretroviral medications can be individually loaded with nanoparticles to successfully fight HIV infection due to new pieces of data and their physicochemical properties.

Recent research has demonstrated the effectiveness and activities of antiretroviral drug-loaded NPs in comparison to free antiretroviral drugs by attributing a 50-fold increase in antiviral effects and a 50-double curtailment in the 50% inhibitory concentration to the HIV inhibitory ability of these treatments.247.

When NPs were first introduced in combination with antiretroviral medications, some research revealed their toxicity and side effects, indicating that NPs would not be able to fully resolve the toxicity problem. According to this recommendation, the NPs should be developed taking into account the morphometric evaluation and structural architecture of particular organs or tissues.

Thus, biological barriers, drug transporters, release time, duration in the body, route of administration, and delivery techniques all play a vital role part in attaining sufficient bioavailability and medication efficacy.

CONCLUSION AND FUTURE PERSPECTIVES

Early research on how antiretroviral medications affected sperm (and testicular tissue) was based on rodent models, but fresh information is now showing how antiretroviral medications affect human testes in a variety of ways, Furthermore, among HIV patients receiving antiretroviral therapy, problems with sperm defects [269, 270], viral replication, and medication resistance271, 272 are becoming more prevalent. The low medication concentration in the sanctuary sites and inadequate delivery to provide a competitive edge in preventing viral multiplication and attaining therapeutic efficacy are partially to blame for these problems.273 Because the entire body is exposed to numerous medications at high levels, the literature has also documented negative and toxic effects of these antiretroviral medications, or HAART. doses. In order to achieve targeted distribution to anatomical sanctuary areas, such as the testes, nanotechnology must be investigated.

Nonetheless, the mainstay of HIV infection management has been lowering the viral load to enhance the quality of life for sick individuals. Drugs can now be efficiently delivered to these sanctuary locations via nano-delivery devices, which can help prevent viral multiplication, rebound, and the negative effects of antiretroviral medications on testicular morphology.283. Thus, nanomedicine has provided a short-term advance in this area. Due to their capacity to enter the brain and the testis, which are known to be "anatomical sanctuary sites" and have historically been difficult to enter, particularly for antiretroviral medications like HAART, nanoparticles are now important in drug delivery. Antiretroviral drug-loaded nanoparticles can now carry a significant amount of high-quality antiretroviral medications to these sanctuary areas because to developments in nanomedicine. However, other researchers have reported that NPs have a variety of harmful effects and toxicities on the body's organs, including the brain, kidney, liver, spleen, lung, testis, and many biochemical parameters. The toxicological assessment and toxicity mechanism of antiretroviral drug-loaded nanoparticles are still poorly understood. Furthermore, it is getting harder to distinguish between the toxicities of nanoparticles, the side effects of antiretroviral medications, and HIV infection sequelae. Future studies on the morphology of the particular organ of interest should be carried out in order to build antiretroviral drug-loaded nanoparticles that decrease the toxicity profile while still achieving drug delivery efficacy. Additional research is also required to confirm the reasons why antiretroviral drug-loaded nanoparticles are harmful and comprehend their toxicity mechanism in detail. It is imperative that a stereological animal experiment be conducted to assess the toxicity of testicular shape and BTB in the nano delivery of antiretroviral medications.

Conflict of interest : none

Orcid:

Hari Prasad Sonwani https:// Orcid.Org/ 0009-0001-8919-7684

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