Assessment of Toxicity and Safety Profiles of Nanoparticles

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Abstract: The world of medicine explored the use of nanoparticles in therapeutics in the last two decades. Owing to the advantages nanoparticles offer, they are proving beneficial to overcome many drawbacks faced by small drug molecules. Since the nature, architecture, shape, size, and mechanism of action of nanomedicines totally different from regularly used drugs, it is important to work on the possible toxicity these nanoparticles are causing so that its safety can be ensured. In today’s scenario, a lot of industries and institutes are synthesizing nano drugs, so it is important to check its toxicity and safety evaluation under in vivo and in vitro conditions, as it has come to fore that number of metal and carbon-based nanoparticles, although proving useful further display increased toxicity. Taken into consideration nanoparticle toxicity and safety, the present review discusses the exact working of nanoparticles at the molecular, cellular, and physiological level and the toxicity associated with it. The present strategies for safety assessment have also been reviewed. The research involving nanomaterials in therapeutics demand strict regulation in nanoparticle synthesis, its usage, properly regulated clinical trials ensuring safety assessment.

Keywords: nanoparticles synthesis; nano drugs; safety regulation; nanotoxicity.

1. Introduction

The term “Nanomedicine” denotes the use of nanoparticles for therapeutic and diagnostic applications. The nanoparticles are mainly synthesized by using a number of metal precursors, biomolecules, and polymers such as proteins [1], polymers [2], metals [3, 4], lipids [5, 6], and carbon. The use of nano-based drugs has been demonstrated to cure a number of diseases such as diabetes, cancer, infectious diseases, and allergy [7, 8]. In success story, now the number of nanoparticles set in clinical trials and approved for its use [9, 10]. In countries like the USA, China, more than 12 Liposomal drugs are clinically approved, while several are taken into clinical trials for evaluation [11, 12].

The features of nanoparticles as a drug explored further since nanoparticles able to showcase electrical and optical properties, which has promising importance in therapies. In a success story, Researchers have successfully used nanoparticle in combination with external energy. It thermally ablates the targeted tissues. The reports published were gold nanoparticles used to be useful with infrared light [13] as well as with radio waves [14], and another report, the use of iron oxide nanoparticles with a magnetic field, able to generate heat has been demonstrated [15].
Nanoparticles also protect themselves from early degradation and ensure sustained and controlled drug release. The advantage of nanoparticles lies with their ability to accumulate drug in the targeted tissue, which justifies its use for better actions. The action of any drug has to face a number of body barriers so that drugs can function at the desired location [16]. The main barriers in drug deliveries are enzymatic and mechanical degradation, renal clearance, vascular endothelium, extracellular matrix, cell membrane, the lysosomes, and membrane pumps. These barriers are promisingly handled by the nanoparticles and reported profoundly efficient by in vivo studies [17].

By considering all these advantages, the use of nanoparticles in disease treatment certainly finds a better place. The increasing research on nanoparticles, especially in nanomedicines, now demands its safety assessment so that its potential toxicity for nanotherapeutic could be put forward. The nanoparticles are unique in its shape, size, and make, and hence those cannot be tested with conventional methodologies generally used for checking bulk molecules; hence, it is imperative to develop stricter guidelines for the approval of nanoparticles once we considered with other molecules. The current paper reviews the key work on nanoparticles toxicity, its effect with systemic response and safety assessment.

2. Assessment of nanoparticles for toxicity/nanotoxicity

The use of nanoparticles in the medical field has reported showcasing great promise, but the toxicity associated with many nanoparticles, as reported by many studies, has restricted its usage and a strict vigilance is the need of the hour to prove its safety. In a study, the use of iron oxide nanoparticles registered harmful effects under in vitro and in vivo conditions, and this negative effect is related to its ability to produce reactive oxygen species [18, 19]. In one study, silver nanoparticles are also registered with cytotoxic effects [20, 21]. It is further challenging with nanoparticles as its toxicity has been linked with its size. For example, gold nanoparticles having a diameter of 1.4nm registered its toxicity, whereas the same nanoparticles having 15nm size did not display any toxicity [22, 23]. The study also showcased that NPs are becoming toxic to the tissue under different shapes and morphology as recorded with carbon nanotubes [24].

However, nanoparticle cytotoxicity and hazardous effect have been well addressed to use it in targeted cell killing of diseased tissue, specifically in the case of tumors and cancers. Provided nanoparticles must be featured with selective targeting so that such a drug could be put forward. The modification of NPs, especially its surface, also reported promising to reduce its toxicity yet to make them effective [25]. In one such report, the involvement of the hydroxyl group to gadolinium fullerene nanoparticles able to reduce its toxicity since it has prevented ROS generation [26, 27]. The success stories also reported when iron oxide nanoparticles coated with a polymer which has improved cell viability by reducing NPs toxicity [28].


The research is going on that how drug targeting to specific tissue can be improved and reduces its toxicity at the same time. Nanotherapeutics associated nanoparticles with drugs as a preferred choice. The success of nanotherapeutics underlined by reduced toxicity than their free-drug counterparts. The first drugs were approved as nanomedicine marketed as Doxil by the USFDA. The drug is having the origin of the liposomal formulation of doxorubicin. The drug is preferred over it free-doxorubicin since it is useful in reducing cardiotoxicity [29].
major research reports are indicating with associated with nanoparticles and drug delivery when the drug side effects were lowered down its indicate the improvement in the accumulation of the drug in the targeted tissue, which is again ensuring the reduction in toxicity and doses with better therapeutic efficacy. As demonstrated earlier, that once injected drug accumulates as only 0.01% in targeted tissues [30]; in contrast, the use of a drug with NPs improves its bioavailability to 1-5%, making it a big success [31].

The success of NPs also been guessed since those cannot freely pass through the vasculature of any tissue easily, while small molecules can; this feature is utilized for increased deposition of nanoparticles in tumor tissue to enhanced permeability and retention (EPR) effect. The EPR effect does exist between the vasculature of tumors and normal tissues [32, 33].

The toxicity of the drug further reported being reduced in the presence of nanoparticles once they were used as an alternative for a stabilizing drug agent. The NPs are more effective once drugs are of class belongs to hydrophobic therapeutics [34–36]. Since in medicine, the number of drugs belongs to water-insoluble categories and to make them dissolved, a number of harmful solvents are used. These solvents are toxic in nature, as confirmed by in vivo and in vitro testing. In one such example, the use of dimethyl sulfoxide for drug testing with cell culture is common [37]. However, DMSO turned out to be toxic to the tissues and cells under in vitro and in vivo studies [38–41]. In a similar manner, the number of hydrophobic drugs is dissolved in toxic solvents such as polyethoxylated castor oil, dehydrated ethanol mixture [42], and polysorbate 80 [43], and many others. These hydrophobic drugs in the future could be replaced with NPs so that rising toxicity could be controlled.

2.2. Toxicity of different nanoparticles.

The dendrimers are highly branched, monodisperse, 3D macromolecule characterized organic nanoparticles used as nano-drug delivery vehicles to carry specific sizes and shaped drug molecules. The dendrimers exhibit hydrophilic periphery and hydrophobic core and are found to be useful in drug loading features [44]. Its real use evidence as a dendritic polymer that able to improve the doxorubicin delivery along with many cytotoxic drugs to solid tumors. The application of dendrimers as a carrier is to reduce the drug load towards noncancerous cells/tissues, and the toxicity of dendrimers against hemolytic cells was reported and found to be toxic [45, 46]. The toxicity of these nanoparticles was reported low with anionic dendrimers and with modified dendrimers since they can mask peripheral cationic groups. These NPs able to minimize hemolytic activity. This gives us a direction that surface engineering or dendrimers will improve its utility by reducing its toxicity, making them safe for use, especially in the biomedical field.

The application of nano drugs has improved and made it to the market as a drug carrier also. Recently, the toxicity concern about these nanosized particles grown to a high level. The particles proving to be a major toxicological concern were noted below 100nm [47, 48]. These small size nanoparticles (<100nm) can easily get internalized by any cell via pinocytosis, which is a real concern. Instead, nanoparticles >200nm only gets internalized by macrophages, causing limited cell toxicity. This phenomenon indicated that small nanoparticles could access any cell of the body, by which increased toxicity potential of them cannot be ruled out. The exposure of nanoparticles via the oral route is reported to be more severe since they showcase higher C_{max} and short T_{max}, along with higher and more rapid systemic exposure of drugs, which gives many severe side effects [49].
The better efficiency of biopharmaceutical drugs and/or its therapeutic index use of lipid nanosystems, mainly in the form of liposomes and solid lipid nanoparticles, is recommended [50]. These types of lipid nanosystems offer many advantages since they are well tolerated by living systems since they are produced from common physiological compounds. This ensures that metabolism should decrease the risk of acute and chronic toxicity [51].

The development of self-emulsifying drug delivery systems providing a new way of drug delivery system, ensuring great safety once we are interested in lipophilic drugs [52–54]. The SEDDSs are further classified as self-micro emulsification drug delivery systems (SMEDDSs) or self nano-emulsification drug delivery system (SNEDDS), which are purely based on the size range of their droplets [55]. Here SMEDDSs ranging between droplet size of 100 to 250 nm and with finer nano-emulsions, of diameter less than 100 nm named SNEDDS. The success of SEDDS evidenced with a product as a dry emulsion of cyclosporine A useful for Asthma treatment, allograft rejection, and for chronic obstructive pulmonary disease making it a future drug delivery prospect [56]. Liposomes are able for encapsulation and deliver both hydrophilic and hydrophobic substances afflictingly after conjugations with polymers. These combinations assure attenuating the opsonization of the liposomes and rapid clearance. The use of PEG-modified liposome is on the rise with the advantage of a significant reduction in non-specific RES uptake [57].

3. Safety assessment of nanoparticles

The efficient use of NPs in nanomedicines could only be ensured its safety assessment is assured at different concentrations, shape, size, and mechanism of action. However, when we are looking at the methodologies available with us to date, these tests or protocols are specially made for the large or small molecules, but those do not ensure it will work equivalent with nanoparticles. In today’s scenario, we are testing promising drug candidates under in vitro and in vivo protocols, and then only the approved drug comes into the market. In a recent scenario, since we are synthesizing nanoparticles for therapeutics, its safety assessment is a prerequisite. However, NPs behaves differently than small molecules in terms of its shape, size, surface properties, and molecular dynamics. The incidences came to force that NPs can sediment, aggregate once interacts in a different solvent, and that changes in molecular dynamics. These properties make them a tough molecule to analyze under testing conditions, and many times does affect analyzing result in a better way. In one such report, where NPs are mutagenic under mammalian assay, but a similar incidence did not record with the Ames test where bacteria used as a screening biological host [58]. The suggested failure of the use of Ames looks at with NPs linked with their incapacity to penetrate the bacterial membrane [59]. But this report remains questionable since NPs are reported to pass on bacterial membranes once traced inside the bacterial cell, indicated that the failure of the Ames test depends on other factors. In confirmation of the study, NPs can interact with tetrazolium salt in the MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide has been reported, thereby interfering in accurate measurement cannot be ruled out [60]. This interaction of NPs with MTT chemicals found to be affecting its toxicity, and it is reported to reduce by 60%.

In addition to the selection of proper methods for NPs assay, it is most important to gather information about its dosage of testing, as in many studies use of NPs in high concentrations has been reported with in vitro conditions. Since the same concentration is not achievable under in vivo conditions, hence giving false information about its toxicity been
tested [61]. As it is estimated that under 2D culture testing, brain microglia cells reported to be damaged with 25µg/ml of titanium dioxide [62], but if these NPs made available at high concentration still it will not damage even epithelial cells in the lung since those will not be exposed to this dose [63, 64].

The incidence of utilizing a high dosage of NPs and resultant its carcinogenicity is mainly recorded since that concentration cannot be applicable under in vivo conditions; for example: - carbon nanotubes prove carcinogenic once given intravenously at a concentration of ~3mg/dose (62), which is equivalent to the 10g/human. That means even at this concentration, blood glucose levels are also reported being toxic [65]. Once inside the body, it is assured that NPs are acting uniformly at a given dose every cell type, but it is not so. In a real sense, a macrophage will recognize NPs and tries to engulf as much as it can, making it less available to other cell types. Hence, it concludes that it is tough to correlate the results of in vitro test with in vivo conditions. Further, once we are synthesizing nanoparticles, it remains with traces of impurity, which is not getting separated or when attempted to do so, making them a hazardous combination to use [66, 67].

### 3.1. Safety features of nanoparticles.

By taking a keen interest in nanoscience and by considering the huge potential in the field of nanotechnology, everyday new nano-compounds are getting synthesized in the academic and industrial sectors [8, 50, 68]. As come to force, ‘nanoscience’ deals with the manipulation of materials at atomic, molecular, and macromolecular scales. These nanostructures are built to assess its unique application at the nanometer scale [69–71]. In the hunt of better molecules- researchers are searching utility in nanoparticles, especially in fields such as electronics, medicine, food science, biotechnology, and pharmaceutics. The success stories are many with the development of nanofillers, nano diagnostic kit, and nano drugs also [50]. Looking at these prospects, now time must come that assess its safety measures, and new methodologies need to be searched out so that better assessment could be carried out. As it has confirmed that nanoparticles can bring many cellular changes such as oxidative stress, inhibition of cell division, cell death, and genetic damage, it is imperative to consider this before testing in any formulation. Further, NPs are variable in features with respect to its size, surface, and chemical composition, which always remain the concern [71–73] taken into the consideration of blooming features of nanotechnology now its safety assessment branch known as nanotoxicology, came to the fore to strengthen its fate in the field of applications.

Solid-lipid nanoparticles set as an alternative to the liposome formulations having many advantages such as a) systematic release of the drug, b) low-cost production, c) ease of mass production, and d) improved physical stability for use. In addition to advantage, several disadvantages are also reported, such as a) limited loading capability, b) relatively higher water content of the dispersion, c) drug expulsion after recrystallization [74]. These drawbacks are overcome by linking peptide, small molecules, proteins to the solid lipid nanoparticles so that it improves pharmacokinetic behavior [75–77]. The use of micellar nanoparticles, which is a spherical nanostructure, has improved its share in drug delivery research [78, 79]. These nanoparticles are mastered to trap hydrophobic drugs and then assure improved absorption and distribution of internal drugs by avoiding opsonization and phagocytic clearance by RES uptake [80]. The use of micellar nanoparticles registered many successes in drug delivery studies such as paclitaxel [81], anticancer drugs NK105 [82] but also suffered from few disadvantages such as rapid elevation of systemic exposure and toxicity of the surfactant.
In the use of the recent advances of polymeric nanoparticles, having a size range between 10-1000 nm able to encapsulate drugs inside a polymeric matrix, which makes them potent to protect the drug from enzymatic and hydrophilic degradation [83]. The polymeric nanomaterial is able to release drugs to the target tissue by desorption, nanoparticles erosion, or via diffusion through the polymer matrix. The commercial uses of polymeric nanoparticles are now employing biodegradable carriers conjugates with proteins, DNA, and RNA-based drugs with less toxicity, and this type of finding may be open new possibilities for researchers in the field of toxicity [84]. The success of polymeric nanoparticles lies with the coating with nonionic surfactants, and resultant presence of surfactants marked by reduced immunological interactions, mainly opsonization. The reduction in interaction between drug chemical groups occurring via Vander Waals forces, hydrogen bonding, and hydrophobic interaction has been reported as a success of these nanoparticles [85, 86].

In the present research era, we are gathering huge information about nanoparticles since they are easy to synthesize yet tough or time-consuming to actually understand its environmental and safety factor, which we are ignoring for the most of the time in today’s research where we are testing the efficiency of nanoparticles for diminishing toxicity of chemotherapy drugs or other drugs. On the other hand, some NPs themselves, proving to be cytotoxic, an allergic compound, or bring about inflammation [87]. Another major effect of NPs lies with their ability to produce reactive oxygen species (ROS) and free radicals. These effects are proving negative to bring about DNA damage, multi nucleus formation, inflammatory events, and fibrosis [88]. The nanoparticles are also proving toxic when those are accumulating within the cells; this situation arrives once those are given in a high dose or exposed for the long term. However, the upper size limit for it has not been ascertained but reported to be ranging between 65 nm and 800 nm [89]. It is further put forward that nanoparticle toxicity remains complex and multifactorial in the feature. The toxicity also remains dependent on the shape, size, and surface properties, mainly the charge, area, and reactivity; it is further confirmed that the size of NPs is most important for toxicity than its composition. The role of nanoparticles surface area also remains useful to predict its toxic and pathological response to particle mass dose [88]. As per shape and size investigation, NPs having needle-shaped carbon nanotubes, nanofibers, and nanowires proving more lethal that brings fibrotic lung disease and rare tumors [90]. The polycationic nanoparticles are also proving toxic to bring about necrosis and apoptosis, and hence while using them as nanocarriers, it is vital to consider their safety. The nanoparticles bring about surface reactivity by bringing about chemical damage to the surrounding tissues. As recorded micron-sized particles once get deposited in the central airways, it does chock lung periphery, causing greater inflammation in that part [91]. The application of nanoparticles is on the rise once they are utilized in cosmetics, stain-resistant clothing, sunscreens lotions. Taken into consideration, a number of researches put forward the minimal toxicity of NPs with skin, and it remains dependent on skin penetration and skin condition also. In some contrasting result, nanoparticles proving to be cytotoxic, and events of proinflammatory to dermal cell lines recorded under in vitro conditions [87, 92, 93]. This study demands the safety of nanoparticles under in vivo conditions to clarify the chronic dosage of nano drugs [87]. In success stories, systemic toxicity of nanoparticles confirmed by acute toxicity assay mainly via a 50% lethal dose (LD50). In a common investigation, nanoparticles are generally taken up to the RES, and the number of target organs remains a member of RES, such as spleen and liver [94, 95]. In a different application, now the biomedical application of nanomedicine, the NPs get a coating of
biocompatible materials that assist them in escaping from opsonization and avoid the RES uptake. This makes the shift away from nanoparticles from the RES. Hence it is important to understand how the potential of nano drugs as toxic compounds is available within in vivo system. The study should consider the factors like the route of administration, and this will surely assist in better understanding of risk factors associated with nano drugs and nanomaterials in the human body so that in the coming time, we can explore the potential of nano drugs in a better way.

4. Conclusions

Another human-made nano molecules are proving to be the savior of human life. These molecules are appearing very tons the equal to different standard tablets; however, the mechanism of action stays varied. This exchange in motion makes them unique, strong but challenging to look at considering they are nanosized, having recreation structured on charge, shape, measurement, and activation with different molecules. Since we additionally recorded that nanoparticles additionally work otherwise in mixture with biomolecules, it is as soon as once more will become precedence look at as soon as it goes into the machine of the host. As it comes to the fore that quantity of nano drugs like carbon and steel produced proving to be eliciting an immune response, amplify ROS generation, cytotoxic, and worried in cell compartment disruption. In the view, the current evaluation mentioned how researchers are discovering new methodologies, following scientific trials to display screen these new nano pills to discover the opportunity of toxicity and protection for future nanomedicine use.

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**Conflicts of Interest**

The authors declare no conflict of interest.

**References**


K. Use of Biofabricated Silver Nanoparticles


