International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 5, p. 156-176, 2019

INNSPUB

REVIEW PAPER OPEN ACCESS

Why Brine shrimp (*Artemia salina*) larvae is used as a screening system for nanomaterials? The science of procedure and nano-toxicology: A review

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Key words: Brine shrimp, *Artemia salina*, Nanomaterials, Toxicology, Lethality assay.

http://dx.doi.org/10.12692/ijb/14.5.156-176 Article published on May 02, 2019

Abstract

Numerous creative key innovations in the field of nanotechnology have turned the minds of scientists to explore new possibilities in diagnosis and treatment of incipient diseases. Nanotoxicity, the sector of vigorous research, has emerged the increased use of nanoparticles mainly in commercially available products. The risk factors of nanomaterials are still unknown. For this purpose, extensive procedures were used to carry out the toxic effects of these nanomaterials. Recently, many researchers have focused on brine shrimp lethality assay owing to their low cost, convenience, and rapid screening procedure. The species of Brine shrimp, *Artemia salina* is commonly used nowadays in drug discovery to screen the toxic effect of different components. The current review article is focused to elucidate the cytotoxic approach of nanomaterials towards Brine shrimp (*Artemia salina*), their mechanistic perspective, procedure and why scientists use it as a screening system with possible future prospects.

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Introduction

Brine shrimp assay was first proposed by Michael *et al.*(1956) which is then developed by Vanhaecke *et al.* (1981). This assay is an effective tool and used for the detection of various toxic substances. It has been used for fungal toxins, heavy metals, plant extract toxicity, cytotoxicity testing of dental materials and pesticides for a long time (Barahona and Sanchez-Fortun, 1999; Martı´nez *et al.*, 1999). It is an advantageoustool to isolate the biological active compounds from the plant extract (Teng, 1993). Cytoxicity of nanomaterial against *Artemia salina* larvae, nowadays attained the attention of many scientists. This method is very simple, inexpensive and attractive because it requires a small amount of nanoparticles to perform the test. Nanotechnology is a well-established field dealing with manipulation, synthesis and strategy of particles structure. It ranging from 1-100nm approximately in size (Wali Muhammad and Shah, 2018). Due to dynamic chemical and physical properties, nanoparticles can be applied in several fields such as health care (Muhammad *et al.*, 2018), synthetic biology, optical devices and cellular transportations (Mohanpuria *et al.,* 2008). The nanoparticle synthesis can be attained through different approaches. It includes physical, chemical and biological approaches (Dillon *et al.*, 2006;Karthikeyan and Loganathan, 2012). The physical and chemical methods are considered time consuming and toxic while biological is considered safe and non-toxic method. This review summaries the scenario of brine shrimp bioassay procedure and different nanomaterial toxicity effect on brine shrimps (*Artemia salina*) larvae.

Synthesis of Nanoparticles

Nanoparticle synthesis has been achieved using several methods including chemical, physical and biological methods. Some hybrid techniques have also been reported for nanoparticle synthesis. (Fig. 1) represents different methodologies for nanoparticle synthesis. Approaches for nanoparticle synthesis can be generally divided into two distinct groups i.e. the top-down approach and the bottom-up approach (Bali *et al.*, 2006). The top-down method utilizes physical approaches like grinding, scratching, milling and cutting in order to break the bulk material down to nano-size dimensions. In this way, the bulk material is transformed into NPs without involving manipulation on the atomic level (De *et al.*, 2008). Some core examples of physical strategies used in NPs synthesis are laser ablation, (Wu and Chen, 2004) vapor deposition, (Lisiecki and Pileni, 1993) pulsed wire discharge (PWD) (Zhou *et al.*, 2008) and mechanical milling (Tanori and Pileni, 1997). The bottom-up method involves chemical and biological approaches that rely on manipulation of atoms, molecules or clusters to produce NPs of uniform shape and size. Some of the methods used in bottom-up approach includes Chemical reduction, (Song *et al.*, 2004)microemulsion (colloidal) procedures, (Kapoor and Mukherjee, 2003) nonchemical reduction, (Huang *et al.*, 1997) electrochemical, (Mott *et al.*, 2007) microwave-assisted (Panigrahi *et al.*,2006) and hydrothermal (Janafi *et al.*, 2000).Fig. 1 shows some of the chief chemical approaches for NPs production. The biological approach for NPs production relies on living systems like plants (inactivated plant tissue, plant extracts, and living plant), (Sajadi *et al.*, 2016; Shankar *et al.*, 2004a) enzymes (Willner *et al.*, 2006) and microorganisms. (Klaus *et al.*, 1999; Nair and Pradeep, 2002). Biosynthesis of metal NPs by plants is also underuse(Shankar *et al.*, 2004b). Physical and chemical approaches result in good NPs yield with defined shape and size but the overall process is complicated and cost inefficient. Moreover, these processes lead to the production of toxic wastes that can be detrimental to the environment. This nullifies the scope of NPs in area like health, medicine and drug delivery. These drawbacks have outdated the use of these approaches for NPs production (Parashar *et al.*, 2009a; Parashar *et al.*, 2009b). This has led to the growing interest of researchers in "Greener Approach" that results in the production of environmental friendly NPs and circumvent the release of toxic compounds into the atmosphere during or after the process (Chauhan *et al.*, 2012; Daniel and Astruc, 2004; Li *et al.*, 2011). One such approach involves capitalizing on biotechnological tools for green synthesis and fabrication of nanomaterials rather than long-prevailing physical and chemical approaches (Joerger *et al.*, 2000) and is referred to as green nanobiotechnology. Green nanobiotechnology generally refers to the synthesis of NPs or nanomaterial through biological routes. The routes being bacteria, (Saifuddin *et al.*, 2009) fungi, (Bhainsa and D'souza, 2006) plants (Bar *et al.*, 2009, Jain *et al.*, 2009; Song *et al.*, 2009) and enzymes (Willner *et al.*, 2007). The byproducts of the aforementioned sources such as proteins (Esteban-Cubillo *et al.*, 2006) can also be implied for the formulation of nanoparticles. The current paradigm shift towards more environmentally benign protocols have turned the 12 standards of green science into insinuating guide for researchers, specialists, technologists and scientific community across the terrene to focus on stable compounds with minimal imperilment (Anastas and Warner, 2000; Kharissova *et al.*, 2013). In the light of the above discussion, green nanobiotechnology can be regarded as a promising area that can be exploited for the synthesis of biocompatible and stable NPs (Narayanan and Sakthivel, 2011).

Bio-based routes for NP synthesis are highly exploited bottom-up approaches in which NPs are being synthesized through simultaneous reduction and stabilization. The whole process involves three basic steps: solvent medium selection, selection of environmental friendly reducing agent and selection of a nontoxic capping material to avoid settling of synthesized NPs (Shankar *et al.*, 2004b, Singh *et al.*, 2011).

Agents of diverse nature have been probed for their capacity to synthesize bionanomaterial with nonhazardous properties suitable for a range of therapeutic applications.

Why brine shrimp (Artemia salina) needed in toxicology experiment?

In-vivo tests are applying on a large number of animal models to check the cytotoxicity with NPs exposure. The routes applied for NPs exposure are injection based, dermal, pulmonary and oral-based(Suh *et al.*, 2009). Due to high cost and time utilization on in-vivo studies, scientists divert to use in-vitro methods for evaluation of NPs cytotoxicity. The use of animals in nanotechnology experiments was criticized by animal rights advocates, the animals should not be used without approval from regulatory bodies such as IACUC (an Institutional Animal Care and Use Committee), as it carries ethical concerns (Suh *et al.*, 2009). To overcome all these reasons in-vitro methods are increasingly applied nowadays. These techniques including XTT assay, MTT assay (Zanette *et al.*, 2011;Sauer *et al.*, 2013), cell culture, the WST-1 assay (Gonzales *et al.* 2010, Hayes *et al.* 2008), BrdU assay, fluorescence Microscopy and LDH assay (Decker and Lohmann-Matthes 1988, Korzeniewski and Callewaert 1983). These assays also carry some disadvantages. As some need solubilization steps with tetrazolium, which is noxious for cell and some needs expensive solvents (Rajabi *et al.*, 2015). There is a dire need of cytotoxicity assay which is simple and hasa rapid screening procedure. Nowadays, the most extensively assay applied in the nano-toxicology study is brine shrimp lethality assay. The species of brine shrimp, *Artemia salina*is commonly used nowadays in drug discovery to screen the toxic effect of different substances (Costa-Lotufo *et al.*, 2005, Manjili *et al.*, 2012, Ramazani *et al.*, 2010). In this case, no sterile techniques are required, and thus *A. salina* assays could replace the more ethically challenging MTT assay that requires animal serum (Mclaughlin *et al.*, 1998). The brine shrimp assay was proposed by Michael and his colleagues in 1959 and was later adopted by many laboratories as a method for preliminary estimation of toxicity (Michael *et al.*, 1956, Vanhaecke *et al.*, 1981). Artemia is great worth test organism as shown in (Fig. 2) and continueused for toxicity testing as it has low cost, convenience and rapid screening procedure (Rajabi *et al.*, 2015).

Brine shrimp lethality assay

Brine shrimp lethality assay is a preliminary and important screening tool for cytotoxicity. This assay monitor toxic substances in plant extract and other based solvents. The toxic substance kills laboratory cultured larvae (nauplii). Nauplii, the first larval stage of many crustaceans, having an unsegmented body and a single eye. It is about 22 mm long. It is enough large and can be seen without high magnification and small enough, which hatch in numerous amount without occupying space in the laboratory. This is a strong bioassay guide which actively employed for cytotoxicity and anti-tumor agents (Sarah *et al.*, 2017). The most important materials and equipment's required to perform the brine shrimp cytotoxic assay includes, table salt, Brine shrimp eggs (a few gram), measuring cylinder (I000mL), spatula, analytical balance, air pump, light source, Magnifying glass, Test tubes, Microtip pipette, Pasteur pipette (Sarah *et al.*, 2017). To perform the cytotoxic assay on brine shrimp, the eggs of brine shrimp are purchased. The eggs are further stored at a temp of 28°C. For hatching of eggs, artificial seawater and light source 37°C are used. This overall method performed in 96 well plates. The newly hatched Nauplii are picked and transferred to each well with the help of Pasteur pipette. To each well, the test sample is added and the volume is adjusted. It leaves for 24 hr and after that, the brine shrimp are taken out from 96 well plates and then counted using a magnifying glass. After 24 hr incubation period, the dead shrimp's percentage is calculated in each well. LD_{50} values are calculated using table curve software (Khalil *et al.*, 2017b).

Literature available on chemical synthesized NPs and Brine shrimp

Chemical synthesized nanoparticles are divided into various categories depending upon their morphology, size, and chemical properties. Based on chemical and physical characterization some of its well-known classes are carbon-based NPs, metal NPs, ceramics NPs, semiconductors NPs, polymeric NPs etc (Khan *et al.*, 2017). The applications of these synthesized nanoparticles in biology or medicine are drug and gene delivery, probing of DNA structure, tissue engineering, tumor destruction via heating (hypothermia), separation and purification of biological molecules and cells, MRI contrast enhancement (Salata, 2004). Various NPs– cell interaction including the impact of NPs on the plasma membrane, and NP-dependent perturbation of basic cellular functions. The possible toxic effects of the nanoparticles are given below in (Fig. 3). Recently, researchers used brine shrimp lethally assay to determine the toxic effect of chemically synthesized nanoparticles. Extensive data are available on these NPs and how they interact with Brine shrimp. Madhav *et al.*(2017) carried out chemically synthesis of CuO-NPs, spherical morphology with an average size of 114 ± 36 nm. This study showed that established NPs are toxic (oxidative stress is induced and major disruptor of proteolytic enzymes) when interacting with *Artemia salina*. Cytotoxic effect was characterized by Graphene oxide NPs with increased mortality rate. This study also emphasized that NPs are in Irregular shape and also presented that its average size is 63.13 nm (Zhu *et al.*, 2017a). Correspondingly, many other chemically synthesized NPs were used against Brine shrimps. A comprehensive study is given in (Table 1) which describes different activities of each NPs towards Brine shrimps.

Table 1. Available Literature on chemically synthesized nanoparticles and their potential towards brine shrimps.

Chemical synthesized NPs	Method of synthesis	Size	Morphology	Dose	Activity	Ref
Polystyrene (anionic carboxylated (PS-COOH) and Cationic amino ($PS-NH_2$) polystyrene)	-	40-50 nm	-	5 to100 µg/ml and 5–100 mg/ml	No signs of mortality but several sub-lethal effects were evident	(Bergami *et al.*, 2016)
multi-walled carbon nanotubes	-	102 nm	anisotropic and fibrous	600 mg/L	Significant Effects on hatchability, mortality, and ethological, Morphological and biochemical parameters.	(Zhu *et al.*, 2017b)
Polyvinylferrocenium supported				0.01to 242.67 mg/L	more distinct toxic effect and	

platinum (Pt/PVF+)	-	-	-		mortality	(Dağlıoğlu and Çelebi, 2015)
Silver	-	30–40 nm	Spherical	2-12 nM	AgNPs increased, the mortality rate, aggregation in the gut region, apoptotic cells, and DNA damage increased in *nauplii* and hatching in decreased	(Arulvasu *et al.*, 2014)
Zn and ZnO	-	40–200 nm	Spherical	10, 50 and 100 mg/L	Zn NPs induced more toxicity than ZnO NPs Mortality	(Ates *et al.*, 2013)
Nickel oxide (NiO)	-	40–60 nm	-	0.2 to 50 mg/L	NiO NPs were caused higher oxidative stress on nauplii and relatively more stable	(Ates *et al.*, 2016)
cobaltoxide (CoO)	-	<100 nm	-	0.2 to 50 mg/L	CoO NPs caused lower oxidative stress on nauplii and dissolved significantly	(Ates *et al.*, 2016)
ZnO-TiO_2	-	45.2 nm	-	0.1to 1.0 mg/L	Significant Effects	(DAĞLIOĞLU *et al.*, 2016)
tin(IV) oxide SnO_2	-	61 nm	-	0.01 to 1.0 mg/mL	Cholinesterase and glutathione-S-transferase activities were significantly inhibited in larvae caused changes in behavioral and biochemical responses, Did not induce any mortality	(Gambardella *et al.*, 2014)
cerium(IV) oxide (CeO_2)	-	50 to 105 nm	-	0.01 to 1.0 mg/mL	Did not induce any mortality of the larvae, Swimming speed significantly decreased in larvae	(Gambardella *et al.*, 2014)
iron(II, III) oxide (Fe_3O_4)	-	20 to 30 nm	-	0.01 to 1.0 mg/mL	Catalase activity and cholinesterase activity significantly increased after Fe_3O_4 NP exposure, not induce any mortality	(Gambardella *et al.*, 2014)
zero valent iron nanoparticles	Precipitation method	200 to 700 nm	S spherical	1, 10, and 100 mg/L	Mortality was demonstrated, Cell membrane damage and bio-uptake	(Kumar *et al.*, 2017)
Au and Ag	Reduction method	-	-	several concentrations	nanotechnology and nanotoxicology, with hands-on experience with basic chemical, material, toxicological, and statistical Principles.	(Maurer-Jones *et al.*, 2013)
TiO_2 and AgTiO_2	-	2 to143 and 1 to 232	TiO_2large aggregates with small pores when compared to AgTiO_2	1 mg/L	AgTiO_2 was found to be more toxic to nauplii compared to TiO_2, The mortality rate in nauplii increased significantly with increasing concentrations and duration of exposure	(Ozkan *et al.*, 2016)

Phyto-fabricated synthesis

Many physiochemical methods were applied for the synthesis of nanoparticles, but they all were seen to have a negative effect on the environment. Plant-based nanoparticles are eco-friendly and its synthesis process is so simple. The metal salt is combined with plant extract and left for some time. The reaction takes place at room temperature. Some reaction took few minutes and some are exceeding to hours. In the end, the solution is reduced containing metal salt into respective nanoparticles as shown in Fig. 4 (Mittal *et al.*, 2013). This type of simple fashionable synthesis attained the attention during the last decades. Moreover, their synthesis is speedy, eco-friendly, cost-effective and can be scaled up easily.

The interface of nanotechnology, plants & brine shrimp

Nanotechnology is a novel emerging domain in different fields which deals with nanoparticles at the nanoscale, which is approximately 1-100 nm in size. Nanotechnology has a tremendous link with biology as a cell of living organisms possess nanoscale molecules like DNA and proteins, which is 2.5 nm and 1-20 nm approximately in size respectively (Anwar *et al.*, 2017; Jain, 2008). Recently, Nanobiotechnology established a novel and promising processes for the discovery of drugs and drug delivery which plays a key role in pharmaceutical industries.

Table 2. List of reported green synthesized nanoparticles and their activity against brine shrimps (*Artemia salina*).

Plant	Part	Synthesized nanoparticle	Size	Morphology	Doses	Activity	Ref
Bergenia ciliata	Whole plant	Silver (Ag) Nanoparticles	35 nm	Spherical	300 mg/ml, 100 mg/ml, 33.33 mg/ml, 11.11 mg/ml		(Phull *et al.*, 2016)
Lantana camara L.	Leaves	Silver (Ag) Nanoparticles	410–450 nm	Spherical	10 µg/ml, 100 µg/ml, 1000 µg/ml	LC_{50}value of AgNPs on A. salina nauplii was found to be 514.50 µg/ ml.	(PS and KS, 2017)
Sageretia thea	Leaves	Zinc Oxide (ZnO) Nanoparticles	12.4 nm	Hexagonal	1 µg/ml, 2 µg/ml, 5 µg/ml, 20 µg/ml, 40 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml	IC_{50} was calculated as 21.29 µg/ml.	(Khalil *et al.*, 2017b)
Moringa oleifera	Flower	Palladium (Pd) Nanoparticles	10nm	Spherical	Different Concentration	50 % when treated (up to 300 µg), revealed it is safe for biological applications	(Anand *et al.*, 2016)
Sageretia thea	Leaves	Cobalt oxide (Co_3O_4) Nanoparticles	20 nm	Cubic	1 µg/ml, 2 µg/ml, 5 µg/ml, 20 µg/ml, 40 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml	The median lethal concentration was calculated as 19.18 mg/ml.	(Khalil *et al.*, 2017c)
Millettia pinnata	Flower	Silver (Ag) Nanoparticles	49±0.9 nm	Spherical	11.11, 33.33, 100 and 300 µg/ml	Nanoparticles showed the cytotoxic effects against (artemiasalina) nauplii with an LD_{50} value of 33.92	(Rajakumar *et al.*, 2017)
Sageretia thea	Leaves	Nickel (NiO) Nanoparticles	18 nm	-	1 µg/ml, 2 µg/ml, 5 µg/ml, 20 µg/ml, 40	IC_{50} value was calculated as 42.60 LG/ ml. A. salina is	(Khalil *et al.*, 2017a)

					µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml	considered as an ideal organism for investigating the cytotoxic potential of a compound	
Sphaeranthus indicus	Leaves	Gold (Au) Nanoparticles	25 nm	Spherical	Different Percent concentration	No mortality showed against *Artemia nauplii*	(Balalakshmi *et al.*, 2017)
Phyllanthus emblica	Seed	Palladium (Pd) Nanoparticles	28 ± 2 nm	Spherical	0.0, 0.5, 1.0, 1.5, 2.0, 2.5 µg/mL	LC_{50} value is 1.25µg/mL at 1.0 µg/mL	(Dinesh *et al.*, 2017)
Ceropegiathwaitesii	*In-vitro* Leaf	Silver (Ag) Nanoparticles	41.34 nm	Spherical	Different Percent concentration	The percentage of mortality rates were significantly increased with increasing AgNPs concentration and exposure time	(Muthukrishnan *et al.*, 2017)
Dictyota bartayresiana	Whole plant	Silver (Ag) Nanoparticles	-	-	Different Doses	(LC_{50}) of brine shrimp nauplii is 196.5µl/l. Toxicity increases with increase in concentration	(Marimuthu Alias Antonysamy *et al.*, 2015)
Bryonia laciniosa	Seed	Silver (Ag) Nanoparticles	~10 nm	Homogeneous spherical	500 µg/ml, 1000 µg/ml	The lower LC_{50} of nanoparticles indicates that it is more cytotoxic than the crude extract	(Balkrishna *et al.*, 2017)
Callistemon citrinus	Leaves	Silver (Ag) Nanoparticles	8-14nm	-	1, 1.5, 2, 2.5, 3 µg/ml	Cytotoxic Activity	(Saad *et al.*, 2017)
Callistemon citrinus	Leaves	Gold (Au) Nanoparticles	5.8-8.84nm	-	1, 1.5, 2, 2.5, 3 µg/ml	Cytotoxic Activity	(Saad *et al.*, 2017)
Artemisia vulgaris		Silver (Ag) Nanoparticles	30 nm	Spherical	100–200 µg/ml	Cytotoxic effect 100% at 200 µg/ml	(Ejaz *et al.*, 2017)

A big data is available in the literature on brine shrimps, which are used for the screening of toxic compounds. The researcher has used a different type of nanoparticles to screen out its toxic or safe effect on brine shrimps. Recently, scientists turned their attention towards the green synthesis of nanoparticles due to its eco-friendly potential. Therefore, the term 'green nanotechnology' is incorporated under the discipline of green chemistry (Wali Muhammad *et al.*, 2018). Twelve different principles are already established by Environmental Protection Agency (EPA), USA in green chemistry for the purpose to reduce environmental hazards which affect human life (Kumar and Yadav, 2009).

Metallic NPs has a reflective impact in the field of nanomedicine and also has a dynamic role in other fields due to vast applications.

These NPs turned the mind of different medical professionals to treat various complex diseases (Khan *et al.*, 2015; Klefenz, 2004). Previously, NPs were synthesized mainly by physical and chemical methods. Due to many disadvantages observed in the traditional synthesis methods, current research focus is diverted toward biological methods that have played a significant role in the synthesis of metal NPs (Arokiyaraj *et al.*, 2017; Emmanuel *et al.*, 2015, Rai *et al.*, 2016).

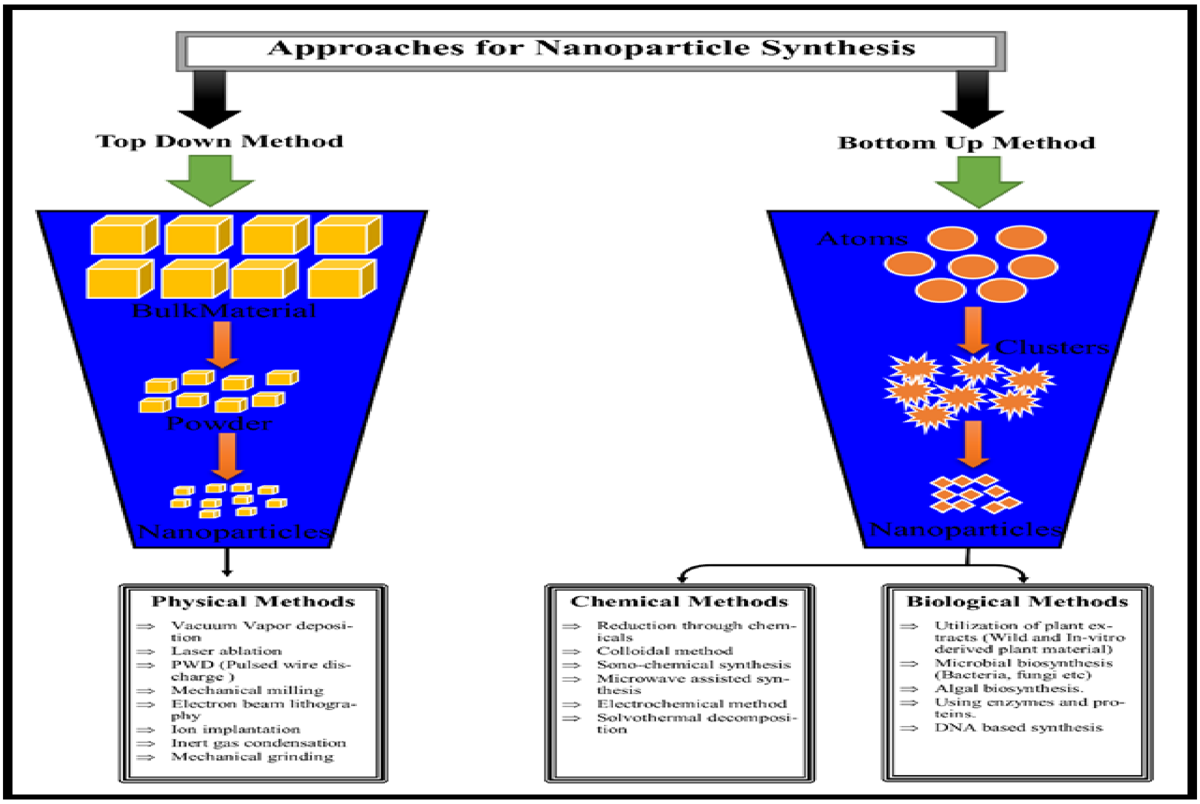

Fig. 1. Different methods used for the synthesis of NPs.

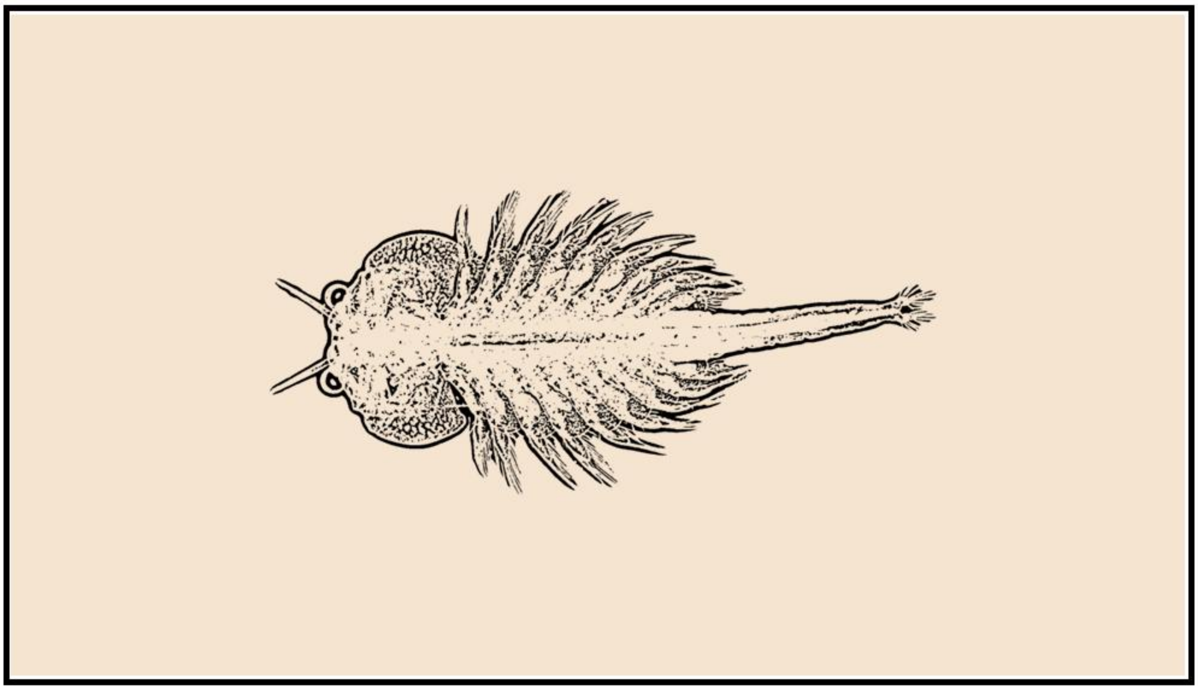

Fig. 2. Brine shrimp (*Artemia salina*).

These methods are based on living organisms like fungi and bacteria, however; plants have provided an ideal platform for the synthesis of biogenic NPs due to their eco- friendly nature (Ovais *et al.*, 2017). Dynamic chemical ingredients are present in medicinal plants that carry the potential to treat different diseases. Hence, medicinal plants can be used for the eco-friendly synthesis of NPs that will control different diseases. Moreover, the In-vitro technique of brine shrimps is used to identify the potential chemical ingredients in these plant-mediated nanoparticles. The plants which are exploited for NPs synthesis in literature and their potential on brine shrimp are indicated in (Table 2) and (Fig. 5). Therefore, plant-based platforms for green synthesis are the best candidates for synthesizing metal NPs which can easily be scaled up for commercial therapeutic applications.

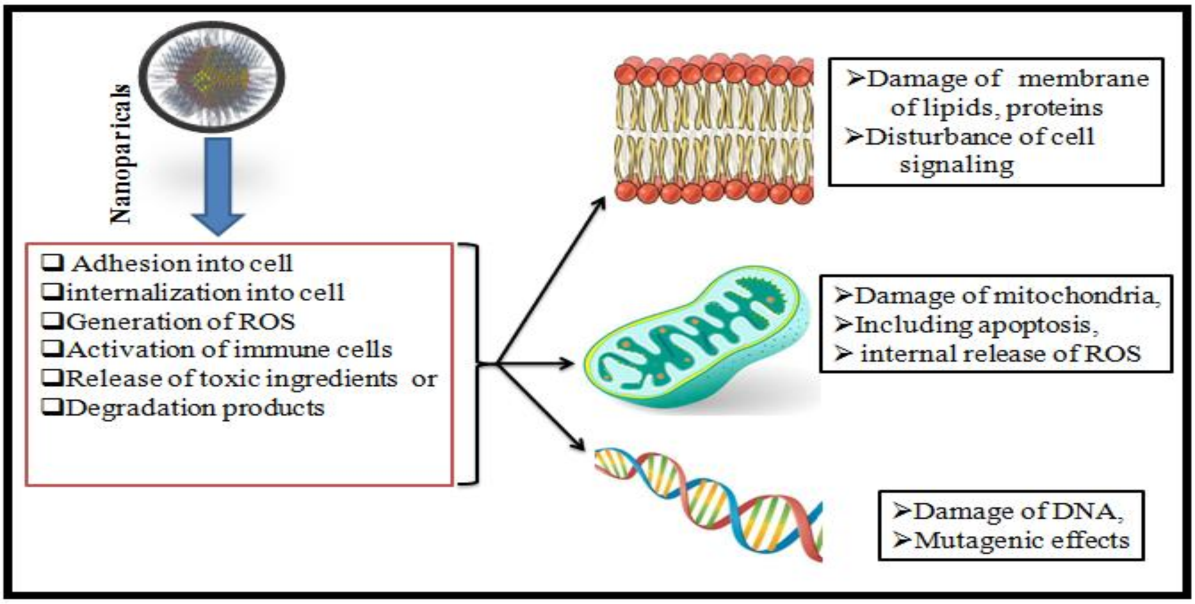

Fig. 3. Scheme of some possible toxic effects of nanoparticles.

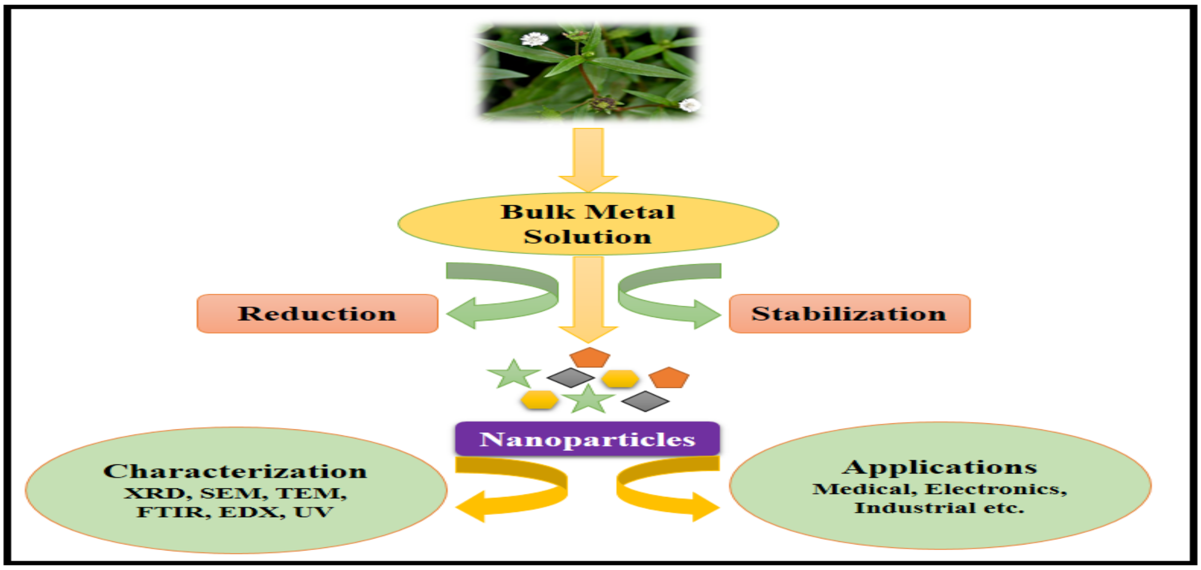

Fig. 4. Green synthesis of nanoparticles from bulk metal solution.

The possible cytotoxicity of green synthesized NPs towards brine shrimps is elaborated in (Fig. 6).

Introduction to nanocomposites and linkage with brine shrimps

Nanocomposites are organic-inorganic multi-phase solid materials where at least one phase has one size range of less than 100 nm (Roy *et al.*, 1986). Nanocomposite materials have emerged as suitable alternatives to overcome the limitations of micro composites and macro composite.

They are reported to be the materials of 21st because they have unique possessing design and also have combination properties that are not found in conventional (Gleiter, 1992).

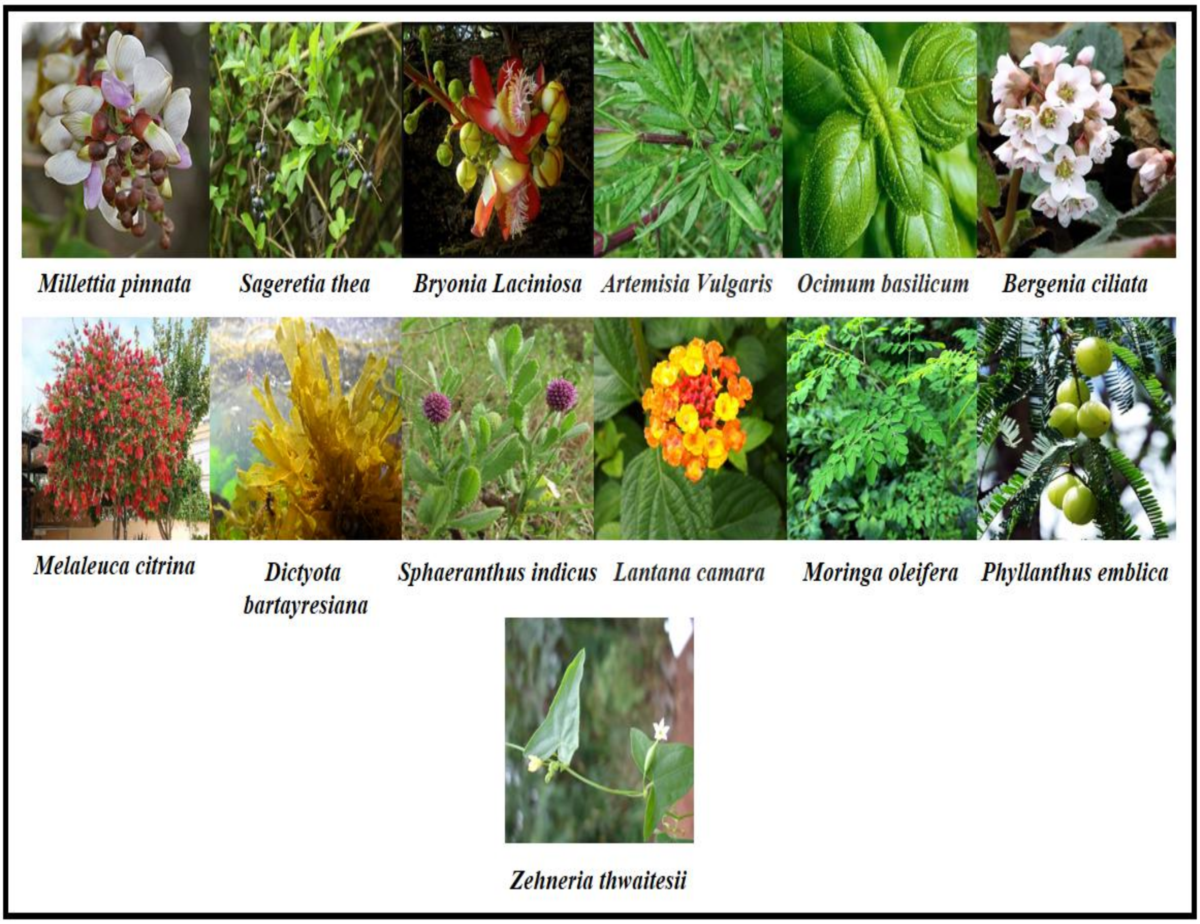

Fig. 5. Different Plants exploited for NPs synthesis and brine shrimp assays.

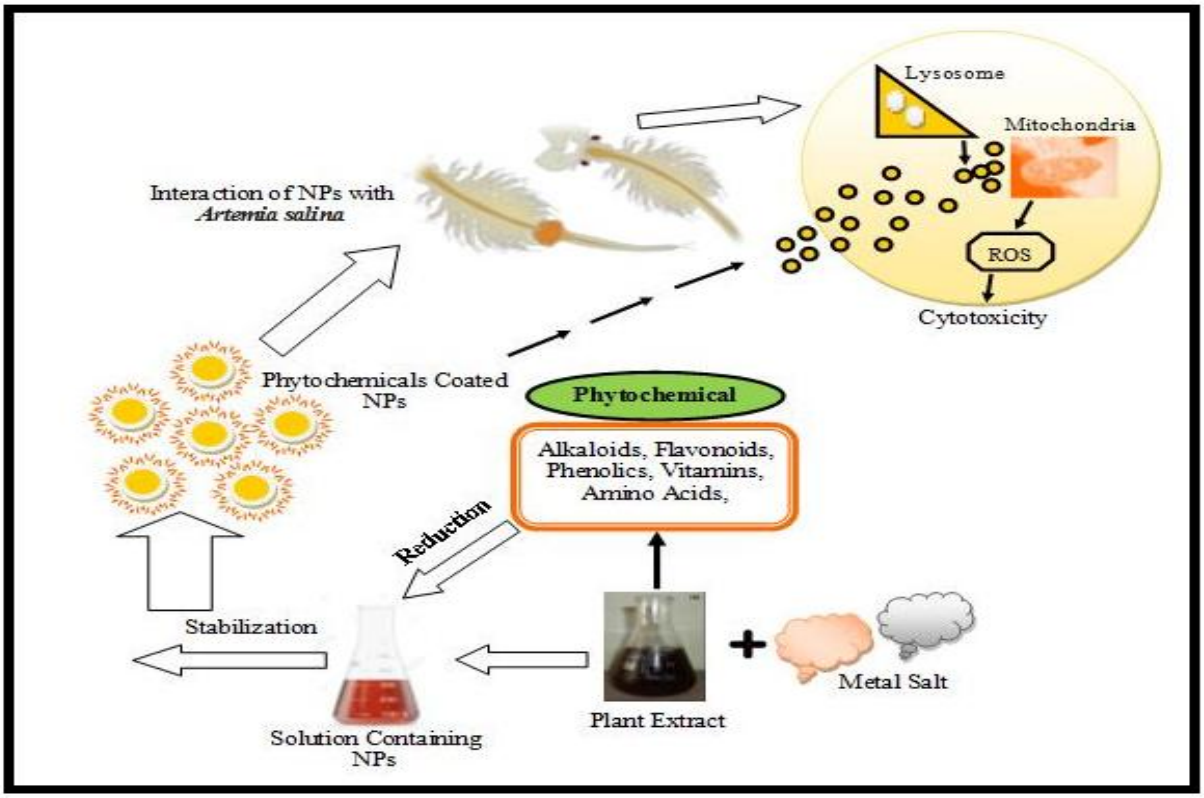

Fig. 6. Diagrammatic representation of green synthesized NPs cytotoxicity towards brine shrimps.

It has been reported that changes in particle properties of nanocomposites can be observed where the particle size is less than a particular level, called 'the critical size. Feature sizes for significant changes in properties reported in nanocomposites systems like Catalytic activity, Making hard magnetic materials soft, Producing refractive index changes, Producing of superparamagneticand other electromagnetic phenomena and Modifying harness and elasticity. Their Feature size (nm) at which changes might be expected is less than 5, 20, 50 and 100 respectively (Kamigaito, 1991).

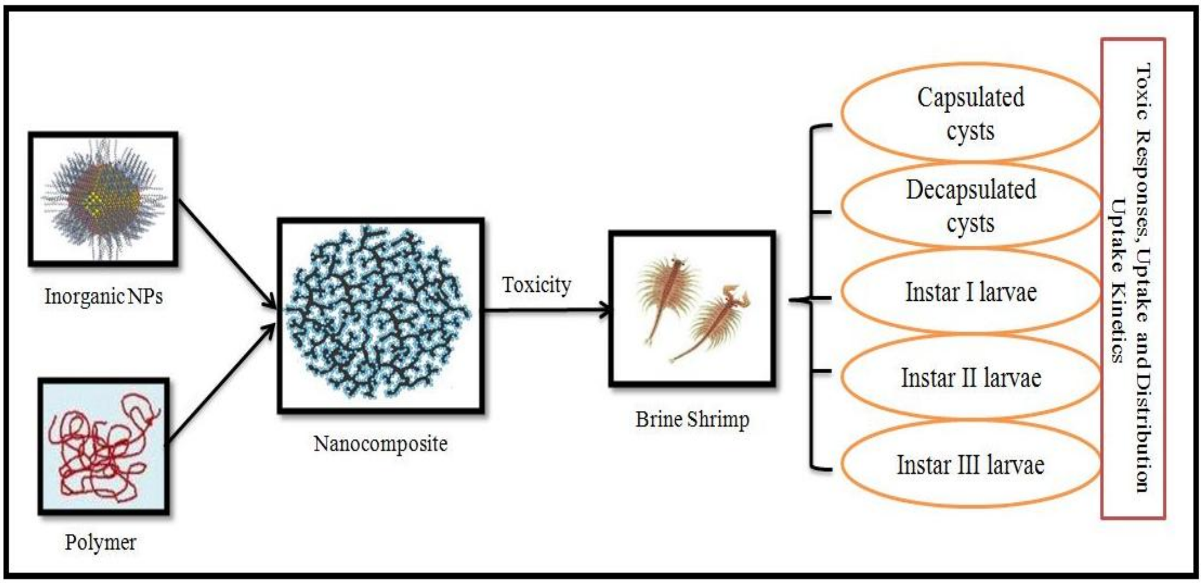

Fig. 7. Scheme of some possible toxic effects of nanocomposite.

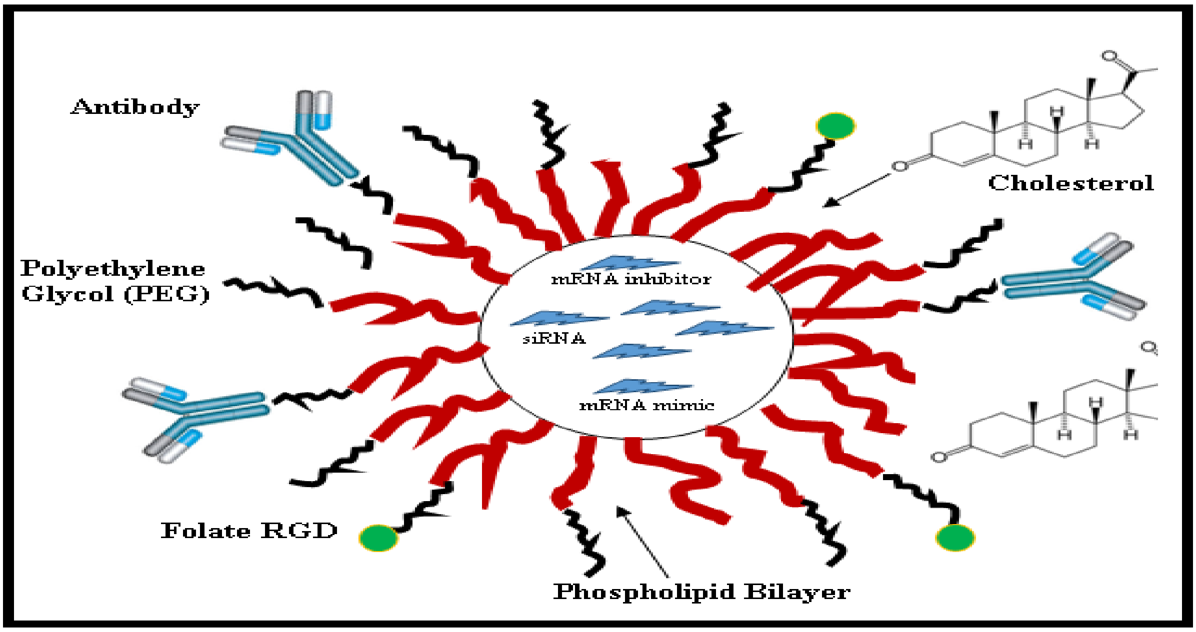

Fig. 8. Showing nanoliposome as a medium for targeted drug delivery.

Moreover, nanocomposite materials can be classified according to their matrix materials in different categories such as Ceramic matrix nanocomposites (CMNC), Metal matrix nanocomposites (MMNC) and Polymer matrix nanocomposites (PMNC).The applications of nanocomposite in various fields such as health safety concerns, bone regeneration, sensors/biosensors, biomedical, catalysis, antimicrobial activity and enzyme immobilization (Ahuja and Kumar, 2009; Allo *et al.*, 2012; Corr *et al.*, 2008; De Azeredo, 2009; Hussain*et al.*, 2006; Liu *et al.*, 2011). The possible toxic effect of the nanocomposite on brine shrimp is given below in (Fig. 7). Chemically synthesized (In-situ oxidative polymerization) Poly (O-Toluidine) (POT)/TiO_2 nanocomposite with spherical morphology showed significant cytotoxicity against brine shrimp (Shakir *et al.*, 2017). *In-vitro* biological brine shrimp lethality assay played a vital role in the toxic effect of nanocomposites.

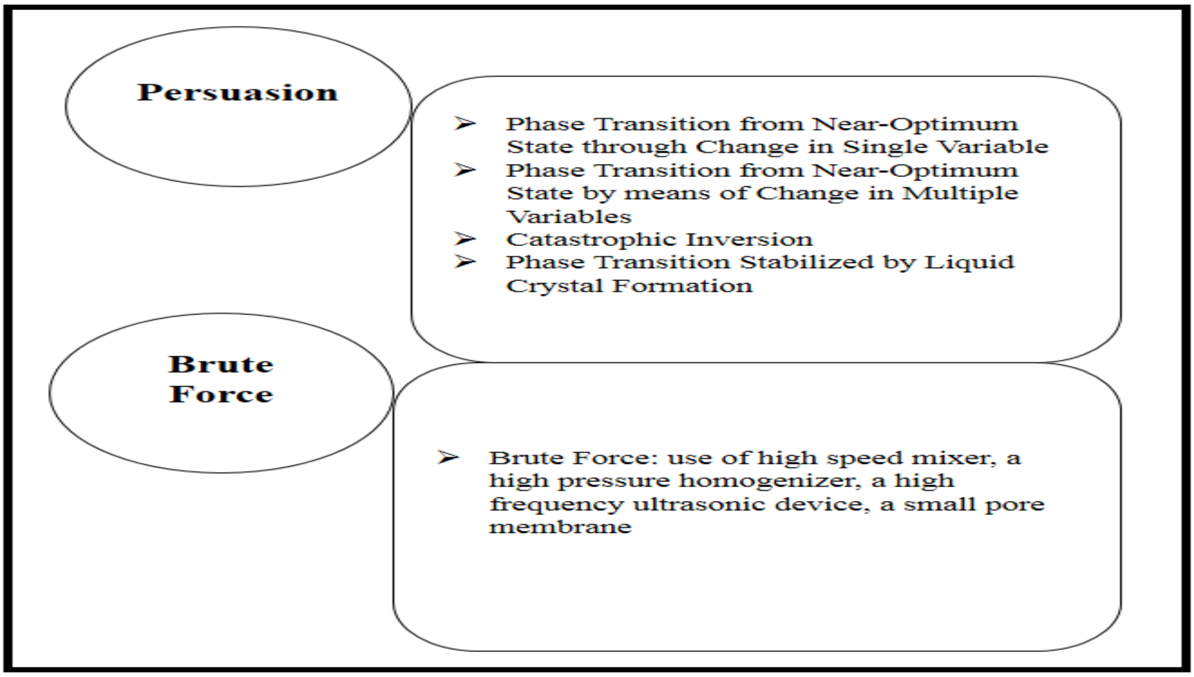

Fig. 9. Essential strategies of the nanoemulsion.

Association between nanoliposomes and brine shrimps

Nano-liposomes, the most utilized targeted drug delivery system, are actually a nano-metric form of liposomes. They can be prepared by simply organizing the array of components i.e., phospholipids and cholesterol etc. (Abreu *et al.*, 2011; Khosravi-Darani and Mozafari, 2010;Ochekpe *et al.*, 2009). About 15 clinically used nano-liposomes are reported currently in treating various maladies (Ochekpe *et al.*, 2009). Different nano-liposomes with therapeutic significance such as DaunoXome reported in the optimal management of blood tumors, Doxil and lipid-dox to be used for Kaposi's sarcoma, breast and ovarian cancers (Chaudhary and Haldas, 2003). The nano-liposomes as a medium for drug delivery has been explained by the below diagram as shown in (Fig. 8). Brine shrimp lethality assay was also used as a medium to verify the toxicity profile of nanoliposomes. Rajabi *et al.*(2015), used lipid-based liposomes having size 139.3 and hence, the result does not display toxicity because the LC_{50} reported was not so significant. It is concluded that brine shrimp lethality assay has a significant role for the screening of toxic components in nanoliposomes.

Available literature on nanoemulsion and brine shrimp interaction

Nanoemulsion is characterized as oil-in-water (O/W) emulsions encompassing droplets diameter from 50 to 1000 nm. They may be of three kinds, for example, oil-in-water (O/W), water-in-oil (W/O), and bio-persistent. The change between these three types can be accomplished by differing the segments of the emulsions (Shah *et al.*, 2010). The Nanoemulsions are additionally named as mini emulsions, ultrafine emulsions, and submicron emulsions. The two essential strategies to set up a nanoemulsion are Persuasion and Brute power as shown in (Fig. 9). The available literature says that brine shrimp lethality assay can be used as a potent tool to screen the toxic effect of nanoemulsions. The results of Islam *et al.* (2017) revealed the cytotoxic effect of phytol nanoemulsion against *A. salina*. The phytol nanoemulsion was found morphologically spherical with the average size of 130-250 nm. In another study, black pepper oil nanoemulsion was found as non-toxic to *Artemia salina* (Swathy *et al.*, 2018). Hence, it is proved that *Artemia salina* can be used as a preliminarily screening tool for nanoemulsion toxicity.

Conclusion

Nanotechnology is a well-established and promising field of the modern era. The toxicity of nanomaterials is still an uphill task to be known. Many methods are being applied to carry out the toxic nature of these materials. Recently, the *in-vitro* Brine shrimp lethality assay convinced scientists to a great extent that they are economical, least awaited for results and easily scale up. Keeping in view the noxious substances screening of the nanomedicines, which play the potential role in various diseases, this assay possesses a pivotal role. Therefore, Brine shrimp lethally assay needs to be utilized for the detection to eradicate the harmful nature of the pharmaceutical drugs being synthesized in the industries on large scale.

Acknowledgments

The authors are thankful to "Khan M" for reviewing this paper.

Disclosure statement

No potential conflict of interest was reported by the authors.

Abbreviations

NPs: Nanoparticles, ZnO: Zinc oxide, PWD: Pulsed wire discharge, Cu: Copper, IACUC: Institutional Animal Care and Use Committee, *A. salina*: *Artemia salina*, EPA: Environmental Protection Agency, AgNPs: Silver nanoparticles, AuNPs: Gold Nanoparticles, DNA: Deoxyribonucleic Acid, CMNC: Ceramic matrix nanocomposites, MMNC: Metal matrix nanocomposites, PMNC: Polymer matrix nanocomposites, O/W: oil-in-water, W/O: water-in-oil, nm: Nanometer, gm.: Gram, hr: Hour

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