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A brief research on current status and development of nano particles in clarified Juice and food assessment studies-A review

Suneetha V*.

Professor and Programme Chair B.Tech Biotechnology, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India -632014.

ABSTRACT

In Recent times Nano particles have been used in juice and food industries without proper basic information about their harm ful nature . Few inventions proved the uptake and disposition of nano particles by the Digestive and Gastro intestinal tract pose problem and there are research investigations where they cross the lining of the Gastro and intestinal tract and circulate in the blood stream, from where they adhere to other body organs and systems. *In vitro* and *In vivo* studies with different types of nanoparticles titanium dioxide, metal/metal oxide carbon nano tubes and silica; but not other nonmaterial's on various cell lines (Hepatic and lung) have demonstrated a variety of oxidative stress-related to immunological and inflammatory reactions. In this study we have postulated that this response is driven by the specific surface area of the nanoparticle and/or its chemical composition , biological synthesis by actinobacteria (microbial),Nano mission etc will be envisaged.

Keywords: Biosynthesis, Nano particle, Biosynthesis, Actinobacteria, toxic nature

Corresponding author

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INTRODUCTION

International status

Recently, many nanomaterials have been commercialized in Juice ,pectinase clarified and allied food industries without proper background information about their harmful nature. Till now the uptake of only a few nanoparticles through different routes have been studied and estimated to be toxic. Few studies have investigated the uptake and disposition of nanomaterials by the GIT and there are reports where they cross the lining of the GIT and enter the blood stream, from where they relocate to other body systems and various organs.

In vitro studies with different types of nanoparticles (nano form of metal/metal oxide, titanium dioxide, carbon nanotubes and silica etc) on various cell lines (lung and liver) have demonstrated a variety of oxidative stress-related inflammatory reactions..

Much of the toxicity data on model nanoparticles have been generated on non biodegradable, non-deformable spheres such as polystyrene and latex or, more recently, the carbon based nanomaterials such as fullerenes and carbon nanotubes (CNT). Recent in-vivo studies have been done on Carbon nanotubes (CNT) [1,3,4,5]. Nearly all in vivo studies on CNT have found histological evidence of inflammation and granuloma formation in rodent lungs. CNTs including multiple walled CNT (MWCNT) and single walled CNT (SWCNT) were able to induce platelet aggregation in vitro and in addition to accelerate the rate of vascular thrombosis in rat carotenoid artery [2,6,7,8]. Carbon nanotubes have also been shown to activate the human complement system via both classical and alternative pathways [7.8]. Recently, PLGA (poly(lactic-co-glycolic acid)) nanoparticles conjugated with alendronate were shown to have an acceptable degree of haemo compatibility following intra venous (IV) administration, suggesting that this material may hold promise as an IV drug delivery tool [3,8]. It should be noted that these materials do not reflect the direct use in juice clarification and food industries but they might be used in packaging where it can come in direct contact with food.

Experimental studies involving oral exposure to nanoparticles have been conducted using copper nanoparticles [6,9,] gold nanoparticles [2.11.14], PMMA (poly(methyl methacrylate)) nanoparticles [15] and chitosan nanoparticles[8.10.12.13] wherein they are found to be toxic above a certain dosage. Instead of above mentioned nanoparticles and CNT there are many nanoparticles (e.g. Fe nanoparticles, Cu nanoparticles, Au nanoparticle etc) and other nanomaterials (e.g. nano emulsions and nano fibrils) which are used in food industries but their toxic effects as of yet are unknown. Inflammatory effects and other immunological effects like cytotoxicity, genotoxicity, and mutation or DNA or nucleic acid damage ability of many nanomaterials remain unanalyzed as of today for many nanomaterials.

Also very few studies were found to be based on in-silico analysis for nanotoxicity of given nanomaterials. This study seeks to expand the knowledge about the nanoparticles which are used in food industry but still its toxicity dosage has not been predicted.

National status

In India, many government and private institutions are involved in research of nanomaterials and its potential benefits but very few are in search for its toxic effects. Institutes like CSIR-IITR, Lucknow; CSIR-CFTRI, Mysore and VIT, Vellore etc are among those which are investigating in this regard. CSIR-IITR, Lucknow has evaluated toxicity state of some nanoparticles including C_{60} , gold, zinc oxide and silver nanoparticles only. Dhawan *et al*. evaluated genotoxicity of stable colloidal dispersions of C_{60} fullerenes. Genotoxicity of the suspension was evaluated with a strong correlation between the genotoxic response and nC_{60} concentration, and with observations made at concentrations as low as 2.2 µg/L [16.17.18].. They have also reported that silver nanoparticles can cause more DNA damage than gold nanoparticles. These nanoparticles were tested for their cytotoxicity and genotoxicity on HepG2 cells and were found to be cytocompatible up to 100 µM metal concentrations. Out of the two metallic systems investigated, gold nanoparticles were found to be more cytocompatible than similar concentrations of silver nanoparticles. It has been also demonstrated that at 100 µM, silver nanoparticles cause more DNA damage compared to gold nanoparticles at similar concentrations [19.20]

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The Relevance of Proposed Study

The study is aimed to address some key important questions related to toxicity of nanoparticles. There are many routes for nanomaterials to enter human body i.e. intravenous (IV), gastrointestinal (GIT), oral etc. The proposed study will evaluate in silico (QSAR, QSPR) toxicity of nanomaterials (for different nanoscale and microscale sizes) and its effects on human health and safety by comparing their physiochemical properties i.e. bioavailability and toxicokinetics. It will be further evaluated by in-vitro and in-vivo analysis. There are many online software tools such as CODESSA, CORAL, TOXPORTAL, TOXCAST which can be used to assess toxicity levels via in silico studies. Currently, they are only used to study the path for drug delivery. These software tools can be used for estimation of toxicity for synthesized nanomaterials. Characterization is an important step in order to know the structure and properties of synthesized nanomaterials which can be elucidated by different methods such as dynamic light scattering, AFM, SEM and TEM. The QSPR and QSAR models can be used to assess toxicity once the physical parameters are accessible. The development of a quantitative-structure-prediction-relationship (QSPR) and quantitative structure–activity relationship (QSAR) model to predict the cytotoxicity of various nanomaterials. A QSPR and QSAR is a statistical model that relates a set of structural parameters that describe a chemical compound to its biological activity. These parameters, which are called descriptors, are typically related to the steric and electronic properties of the compound, and they can be computed or measured in experiments.

The interaction of nanomaterials in the human body can be studied through various methods. Possible change in the nanomaterials following passage through the GIT can be done via exposure to an artificial gastric-fluid environment wherein the nanomaterials are mixed with HPNC (hydroxypropylmethyl cellulose) and added to gastric fluid (0.2 % NaCl, 0.32% pepsin, 0.07% hydrochloric acid) at pH 2 and 37°C with a constant stirring of the components. The plasma collected can be analysed by plasma atomic absorption spectrometry. Similarly, different cell lines such as lungs, liver, stomach, colon and blood corresponding to different body parts give an estimate of the Safe Upper Level (SUL), half maximal inhibitory concentration (IC 50) and median lethal dose (LD 50) levels. Further genotoxicity and cytotoxicity of a cell can be evaluated by Comet assay to detect for any genetic damage to the cell by the given nanomaterial. Any amount or quality of DNA damage can be analysed quantitatively by staining with Ethidium Bromide (EtBr) and analysis by image analyzer software, length and percentage of DNA migrated as compared to control, followed by checking for any aberration or gene mutation. The in vitro studies are helpful in estimation of SUL, IC 50 and LD 50 values.

The lethal dose estimated from in silico and in vitro studies can be used to study toxicity in rat model. The in vivo study gives a clear picture of percentage mortality in the form of LD50. Studies will include effects of nanomaterials on various organs such as kidney, liver, stomach, blood, brain etc. Since liver is involved in absorption and distribution, it is the main organ in the study of toxicity levels. The liver enzymes such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), and glutathione-S-transferase (GST), can be analysed by commercial kits along with spectrophotometric studies. Increased levels in these enzymes indicate liver damage and any toxicity. Haematological analysis can be done for any deviation from the control related to haemoglobin levels, RBC count, mean corpuscular volume (MCV), platelet number or shape and any other abnormality in blood cells. Body weight can be compared for any presence of growth remission or loss in weight due to shrinkage of body organs. Comparison of liver, spleen, kidney, heart, and brain with control for any change or enlargement in shape. Histopathological studies will aid in studying the accumulation of nanomaterials in tissues of various organs. Metabolomic study of hepatotoxicity and nephrotoxicity can be done by H^1 NMR spectroscopy

Nanocomposite plastics could provide the basis for strong packages with high barriers to oxygen and water vapour; silver and metal oxide nanoparticles are potent antimicrobial agents that can kill foodborne pathogens. Naturalness may also explain why food-packaging applications seem to enjoy greater support than nano materials added directly to food, given public aversion to molecular-level manipulation of food and the fact that food packages are often composed of materials that are not natural even in the absence of nanomaterials.

BIOLOGICAL SYNTHESIS

NPs of the above methods (physical and chemical synthesis) have many disadvantages like cost, high energy input, and toxicity of the chemicals used, side effects, harmful by-products. Hence, an alternative method by which the above said disadvantages could be overcome has to be developed in order to yield safer products in a cost effective method. Plants, bacteria, yeast, fungi, viruses and algae were reported to synthesis NPs in an eco-friendly method (15). Thus, there is an ever increasing need to develop the high yield, low cost, environment friendly method for the production of nanoparticles and the biological method are much suitable for the nanoparticle synthesis. Both the unicellular and multicellular organisms are known for their capacity of intracellular or extracellular production of metallic nanoparticles. The major advantage in biological synthesis is cost effective and non-toxic.

BIOISYNTHESIS OF NANO PARTICLES BY ACTINOBACTERIA

The marine environment is characterized by the hostile parameters such as high pressure, salinity; low temperature, absence of light etc. and the marine actinobacteria have adapted themselves to survive in this environment. Marine Actinobacteria is an important source of marine environment. Actinomycetes are a group of bacteria which possess many important and interesting features. Actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now recognized as prokaryotic organisms The majority of actinomycetes are free living, saprophytic bacteria. They are producers of a large number of natural products, many of them with clinical, pharmaceutical or agricultural application [1, 20]

Analysis of nanoparticles in food stuffs:

In food sector, nanotoxicology and nanoecotoxicology are still in their budding stage and risk assessments are practically nonexistent. Therefore, progress in nanoparticle testing (*in vivo* and *in vitro*) is urgently needed to guarantee consumer safety, including the development of standard testing materials and testing methods. In addition to toxicity studies, various uptake paths as mentioned in the text above have to be studied, including dermal, oral and intestinal.

These analysis techniques should be able to

(a) Deal with mixture and heterogeneous samples,
(b) Minimize sample alteration to avoid artifacts and
(c) Provide as much information as possible,

Since in most of the analytical techniques the sample gets consumed or destroyed because of the destructive nature of the technique. Hence, conventional techniques such as optical microscope are unsuitable for measuring extremely small size of NPs. Several studies have been reported on the detection of NPs using field flow fractionation (FFF), hydrodynamic chromatography (HDC), and dynamic light scattering (DLS). Although FFF, HDC, and DLS are good methods to detect the size distribution of NPs, they require suspending NPs in solution prior to testing, and it is difficult to apply these methods in detection of NPs in foods due to the difficulty in extracting the NPs from food matrices, especially when the concentration of NPs is at a very low level. In addition, these methods cannot be used to measure important physical properties (shape, etc.) of NPs and the interaction between NPs and plant tissues.

Nowadays, researchers are using TEM and SEM to detect nanoparticles after proper fictionalization. For quantification of nanoparticles present in food stuffs, AAS, ICP-MS, ICP-OES are used. Size exclusion chromatography will be a great tool for these analysis which could be exploited. Method development and method validation is a must activity in the analysis of these nanoparticles in food stuffs. Hence, Good laboratory Practice for *in vitro* toxicity and Good Cell Culture Practice be followed from the beginning. It is promising that some good practices for how to test NPs have emerged from expert workshops, which need to be consulted for precise and accurate analysis of the nanoparticles in food stuffs.

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FUTURISTIC ASPECTS OF NANO FOODS

At this point in time the term nano food does not refer to foods produced directly using nanotechnology techniques. The future could bring dramatic changes in this area.

Nano machines might be able to produce foods molecule by molecule but this is many years away. Future developments in the short term could include packaging that reflects heat to keep ice cream frozen in a hot car, self-healing packaging that repairs itself when perforated and packaging that can change its properties under certain conditions e.g. a milk carton that changes colour if the milk has spoiled. A scientist with Kraft foods, Manuel Marquez-Sanchez, has outlined plans for a nanotechnology enabled drink, "The idea is that everyone buys the same drink, but you'll be able to decide its colour, flavour, concentration and texture".

FOOD NANO-PARTICLE INDUCED GENE EXPRESSION ANALYSIS IN HUMAN INTESTINAL EPITHELIAL CELLS

Usage of nano materials in food and food products, food processing and packaging has increased rapidly in the recent years. Titanium dioxide (TiO2) is a popular whitener which in micro-size level has been approved long ago to be used as food additive (FDA, 1966). Nano sized TiO2 does not have white color and hence used as transparent films in food materials. This prevents confectionary products from melting and improves shelf life of food. It is also used as oxygen barrier or UV absorber in food packaging. Besides TiO2, silver and zinc oxide nano particles are also used in food industry. However, the impact of these nano particles in human intestine, particularly at the molecular level, is poorly understood in the research community. Nanoparticles in the food may play a significant role in chronic inflammatory bowel diseases or bowel cancer. Expression of interleukin-8 has been found to be one of the marker mechanism involved in inflammatory bowel disease. In addition, generation of reactive oxygen species is the main contributor of nano particle induced toxicity. With this background, the proposed work aims at studying the expression of interleukin-8 gene and antioxidant enzyme genes in Caco-2 intestinal epithelial cells exposed to different concentrations of titanium dioxide, oxides of zinc and silver.

Nanoparticles – The nanoparticles (titanium dioxide, oxides of zinc and aluminium) that are used in food industry will be collected. The nano particles synthesized in this collaborative project will also be used as comparison. *Cell lines* – Caco-2 human intestinal epithelial cells will be obtained from National Centre for Cell Science, Pune and maintained in cell culture lab of VIT University, Vellore using standard protocols.

Exposure of cells to nanoparticles – The cells will be exposed to different concentrations of nanoparticles following standard procedure.

Target genes and primer design – Based on the ROS analysis in the cell culture (which will be performed by another collaborator), the antioxidant enzyme coding genes will be selected as target genes in addition to interleukin-8. Human beta-actin gene will be used as internal control. The sequences of these genes will be used to design primers. The oligos will be synthesized in Eurofins Pvt Ltd, Bangalore.

Nucleic acid extraction and analysis – Total RNA will be isolated using Human RNA easy isolation kit (Qiagen, USA) as per manufacturer's protocol. RNA will be isolated from cells exposed to nanoparticles and also in untreated cells. After analysis in electrophoresis, RNA will be quantified and used for cDNA synthesis using cDNA synthesis kit (New England Biolabs, UK) using gene specific primers as well as oligo (dT) primers.

Q PCR analysis – Cyber green method will be used to analyze the expression of these genes in nanoparticle treated cells and untreated cells. Statistical analysis will be performed with enough replications and the data will be interpreted. This project includes process optimization for nanomaterial synthesis and their characterization to obtain nanomaterials of same dimensions. These nanomaterials can be evaluated in-silico by QSPR and QSAR indicating if they are toxic. If they are proved to be toxic then they will be tested on cell lines for DNA damage, genotoxicity and cytotoxicity. The lethal dosage derived from in vitro studies will be given to rat models to study biochemical parameters and histopathology.

The Outcome of Proposed Study

The study will address the adverse effects of commonly used nanomaterials. Strategies to estimate the SUL, IC50 and LD50 for nanomaterials will be an important outcome for this project. An optimized procedure using statistical approach (Response Surface Methodology i.e. RSM) for Nanomaterials synthesis (including NPs, nanofibrils, nanoclay and nanoemulsions) will be another outcome of the proposed work.

The study will give a comparative analysis of the physiochemical properties (charge/mass ratio, surface area by volume ratio, size and size distribution, viscosity, zeta potential analysis, agglomeration state, mass, chemical composition, surface properties, crystal structure, porosity, solubility) and their changes in relation to different production methods of nanomaterials (nanoparticles e.g FeNP, AuNP, CuNP etc; nanoemulsion e.g.neem oil nanoemulsion etc; Nanofibres e.g. globular proteins, liposomes etc; nanoclays in packaging e.g. Durethan etc.) which will be very useful for in silico analysis and also it will be a basis for a theoretical design of nanosensors.

This study also addresses another novel area of computational approach in studying toxicity which is currently being applied to drugs only. QSPR and QSAR analysis can be done by using open access soft wares like CORAL, CODESSA, TOXPORTAL or TOXCAST etc. Integrated approach of in-silico, in-vitro and in vivo approach will provide a basis to classify nanomaterials in different toxicity classes. In–silico analysis indicates the possibility as to whether the nanomaterials are really toxic or they have potential applications in food industry and are safe for consumption. Cell line analysis will give a predictable value of lethal dosage and the possibility of accumulation of nanomaterials in various tissues.

In vivo study will help us to decide SUL, IC 50 and LD 50 for nanomaterials. This will help in comparative analysis of toxicity in between nano and native form. The study of the liver and renal enzymes will give an estimation of lipid peroxidation caused by ROS and the oxidative stress within cells. The body and organ weight will be a measure of nanomaterial accumulation. Morphological study of the mottled surface or necrosis in epithelial cells will reveal about any presence of organ atropy. This provides for an efficient, cost-effective study of the toxicity levels with some positively ethical aspects.

Scope the Application

Despite an increasing application of nanoparticles there is a serious lack of information concerning their impact on human health and safety. Nanomaterials have been commercialized in various food and juice industries The in silico model along with the in vitro model will give a predictable dose level and concentration which can further be tested by rat model and can be used to estimate toxicity in humans.

This study will be helpful in assessing food safety levels wherever nanomaterials will be involved. Many industries are using nanomaterials without proper approvals. Since nanomaterials today are often used in different products,. This study will be useful for researchers and industrialists in order for them to incorporate any nanomaterial in food applications.

Another aspect of this study is to evaluate the toxicology of natural components. The natural compounds such as vitamins, carbohydrates or proteins which are encapsulated to increase the bioavailability of these lipophilic compounds can act as potential toxic agents. *In vitro* analysis through cytotoxicity, genotoxicity and toxicokinetics give us a way to rethink the use of nanomaterials in food. Following our studies industries can fix doses and protocols and thus many diseases and toxic effects due to nanomaterials can be minimized. Kinetic modelling and physicochemical properties, DNA damaging property analysis and further mutagen city will determine deposition and fate of the nanomaterials in lung, liver, stomach, colon, blood (through cell line study) by different routes e.g. dermal, oral, nasal etc which gives important result for risk assessment. Vulnerable members of the population may include those with pre-existing digestive disorders, which may potentially be impacted by the presence of nanoparticles, although on the other hand nanoparticles offer many potential routes to therapies for these same diseases. Nanotechnology is in a nascent stage. The every property that makes it useful for many applications also makes it toxic. The need of the hour is to estimate the maximum concentration to be used in food and juice industries without any adverse effect.

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