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RESEARCH ARTICLE

"ZEIN NANOPARTICLE AS SAFE EFFECTIVE BIOCOMPATIBLE NANOCARRIER".

Shaimaa Mohammed Ewais[1], Rania Hassan Fahmy[2], Laila Ahmed Rashid[3] and Fathia Zaki El-Sharkawi[1].
1. Department of Biochemistry and Molecular biology, Faculty of Pharmacy Helwan University.
2. Department of Pharmaceutics and Industrial pharmacy, Faculty of Pharmacy Cairo University.
3. Department of Biochemistry and Molecular biology, Faculty of Medicine Cairo University.

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Abstract

Zein nanoparticles (ZNPs) is protein nanoparticles (NPs) that can effectively carry wide range of both hydrophobic and hydrophilic drugs. ZNPs was formulated by coaservation method that is considered as very advantageous regarding simplicity and safety as no harmful materials were used in the preparation. The safety pattern of this NPs so that it can be used without any hazard in human is an urgent concern that should be studied deeply. This study revealed that ZNP is a safe NPs with excellent biocompatibility and minimal toxicity toward viable cells that confirmed by invitro cytotoxic analysis of HepG2 cell lines using neutral red assay. In conclusion, our findings suggested that this natural protein nanomaterials is very biocompatible with no toxicity toward viable cells.

Introduction:-

Nanomedicine includes the use of NPs for all medical purposes. During the past two decades, a rising trials in nanomedicines have received regulatory approval and many more show promise for future clinical translation. This created an urgent need to evaluate the safety of NPs in order to achieve biocompatibility and desired activity. However, it is unjustified to make generalized statements regarding the safety of NPs, since the field of nanomedicine contains a wide range of manufactured NPs made from various materials. In regards to clinical application, stricter regulations for the approval of nanomedicines might not be required. So, safety evaluation assays should be adjusted to be more appropriate for engineered NPs (Chen et al., 2016).

The application of natural polymers on colloidal systems facilitates the fabrication of nanomaterials with several attractive features such as high biocompatibility, biodegradability and low toxicity. There are numerous reports on the application of natural polymers such as chitosan, alginate, gelatin, cellulose, collagen, zein, etc as biomaterials

Zein is a group of alcohol-soluble maize proteins extracted from corn gluten meal. Being prolamins, the surface of zein molecules includes more than 50% hydrophobic amino acid residues and is typically dissolved in 70–80% aqueous ethanol before dispersion into water to precipitate zein as NPs, which can simultaneously encapsulate various lipophilic compounds co-dissolved in aqueous. To incorporate zein in aqueous systems, zein can be prepared as dispersible NPs by dispersing a stock aqueous ethanol solution of zein into water , where zein precipitates to form NPs because the mixture with a lowered content of ethanol becomes a nonsolvent of zein. The process can be used to encapsulate various lipophilic compounds that are co-dissolved in the aqueous ethanol stock solution and co-precipiate with zein during dispersion in water (Chen and Zhong, 2015; Shukla and Cheryan, 2001).

Corresponding Author:- Shaimaa Mohammed Ewais
Address:- Department of Biochemistry and Molecular biology, Faculty of Pharmacy Helwan University.

The application of natural polymers on colloidal systems facilitates the fabrication of nanomaterials with several attractive features such as high biocompatibility, biodegradability and low toxicity. There are numerous reports on the application of natural polymers such as chitosan, alginate, gelatin, cellulose, collagen as biomaterials (Kumar et al., 2003; LIM et al., 2006). Among these polymers, zein is the storage protein in corn kernels that has a variety of unique characteristics and functionalities that makes zein valuable in various applications. It is a hydrophobic protein of molecular weight of about 40 kDa and classified as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA). Zein is the major storage protein of corn, it is soluble in an aqueous alcoholic solution, it has amphiphilic character, so it can interact with hydrophilic and hydrophobic drugs and also the hydrophobic regions of zein can cause aggregation into colloidal particles in aqueous solution. The surface charge of zein varies with the pH of the environment, with an isoelectric point (PI) of zein at pH 6.8 (Cabra et al., 2005). It is biocompatible and has degradation products that can enhance cell proliferation (Sun et al., 2005).

Knowledge of the toxicity effects of these small substances is limited, but is rapidly growing. Many studies have shown that some NPs demonstrate toxicity in biological systems. Thus several studies in about their toxicity toward human and the environment are urgently needed. Some researchers have shown that most of the NPs can release active oxygen and cause oxidative stress and inflammation by the reticoendothelial system (RES).

Material and method:-

Materials:-

Zein (protein from corn, pharmaceutical grade F4400C) was purchased from Flo Chemical Corporation, MA, USA., Ethanol, HCl, NaOH, Na2CO3, CuSO4, Sodium potassium tartrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Folin-Ciocalteu; Fluka Chemie GmbH, Buchs, Germany.

Cell culture:-

Human transformed cell lines, from liver (hepatocellular; HepG2), lines were obtained from Vaccera (Giza, Egypt). Cells were maintained in DMEM supplemented with 100 µg/ml streptomycin, 100 µg /ml penicillin and 10% (w/v) heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO2 atmosphere at 37°C. Dulbecco's Modified Eagle's Medium (DMEM); Lonza, Allendale, NJ, Fetal bovine serum (FBS); GIBCO, USA, dimethyl sulfoxide (DMSO) solvent; BDH, England. Neutral Red was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

zein nanoparticles (ZNps) preparation:-

Zein was dissolved in a hydroalcoholic solution at pH 3 then precipitated by dropwise addition of 10 ml of double distilled water (pH 4) while vortexing; so that ZNPs were formed via coacervation, followed by centrifugation for 1 hour at 1000 rpm (Hu and McClements, 2014; Regier et al., 2012).

Invitro cytotoxixity of ZNPs in liver cell lines using neutral red assay:-

The cytotoxicity of ZNPs was tested against HepG2 cells by the neutral red assay as previously described (Repetto et al., 2008). Cells were seeded in two 96-well plates at density 5000 cells/well one day prior to the experiment. Then media were removed and 200 µL 0.0075% NR solution were added to each well and incubated for 2 hours in humidified atmosphere of 5% (v/v) CO2 at 37°C and subsequently washed with PBS. Ethanol/ water/acetic acid (50:49:1) solution was used to dissolve the NR stained cells and colour intensity was measured at 540 nm in a micro-plate reader. IC50 was calculated using MasterPlex reader 2010 hitachia (GIRSS) (Global Institute Research Services and Solutions).

Statistical data:-

IC_{50} of ZNPs was calculated by using the MasterPlex reader 2010 hitachia (GIRSS) (Global Institute Research Services and Solutions).

Results:-

Cell studies were conducted to determine biocompatibility, absorbance values at a wavelength of 430 nm after 24 and 72 hours showed no statistical significant differences between cells that treated with ZNPs and the control (untreated cells) condition indicating no cytotoxicity and good cell biocompatibility (Figure 1).

Figure 1: Effect of ZNPs treatment on cell viability. Cells were treated for 24hrs and 72 hrs respectively with free ZNPs. Viability assessed by Neutral red assay.

Discussion:-

NPs, often defined as materials with two or three dimensions between 1 and 100 nm possess unusual properties related to their size, shape, and chemical composition. Indeed, there is a common assumption that the small size of NPs allows them to easily enter and traverse tissues, cells, and organelles since the actual size of engineered NPs is similar to that of many biological molecules (e.g. proteins) and structures (e.g. viruses). However NPs may not freely or indiscriminately cross all biological barriers but these processes may instead be governed by the specific physico-chemical properties of the NPs themselves as well as the identity of the functional molecules added to their surfaces. Clearly, then, an increased understanding of the mechanisms that dictate the behavior and fate (biodistribution) of NPs upon introduction into the body is instrumental not only for the development of NPs for targeted drug delivery, but also for the prediction of the potential toxicological responses to such nanomaterials (biocompatibility).

The development of NPs for biomedical applications including medical imaging and drug delivery is currently undergoing a dramatic expansion. However, as the range of nanoparticle types and applications increases, it is also clear that the potential toxicities of these novel materials and the properties driving such toxic responses must also be understood. Indeed, a detailed assessment of the factors that influence the biocompatibility and/or toxicity of NPs is crucial for the safe and sustainable development of the emerging nanotechnologies.

There are different techniques for preparing the NPs; the safety of preparation technique and solvents and additives are very crucial in determining the safety of the produced NPs and the stability of the loaded drugs. Here, Coacervation that involve the separation of solutions into colloidal systems with two liquid phases (Bungenberg de Jong and Kruyt, 1929), was followed to form spheres. This separation involves the formation of one phase rich in polymer (the coacervate) and another phase lacking polymer, which is brought about by the partial desolvation of a previously dissolved polymer (Arshady, 1990; Gandera et al., 2013; Madan, 1978). The addition of aqueous solutions results in the necessary desolvation and the formation of a zein-rich nanosphere phase (Hurtado-López and Murdan, 2005). This method has been previously used to encapsulate a variety of drugs (Fu et al., 2009; Liu et al., 2005; SUZUKI et al., 1989; Wang et al., 2005), but here we targeted to assess the safety pattern for this prepared NPs.

The toxicity of ZNPs was evaluated by studying cellular morphology and viability test (neutral red assay) under control and exposed conditions. The cell study indicated no cytotoxicity and good cell biocompatibility of this Nanoparticle formulated by this simple technique that include no as no harmful materials were used in the preparation and also no drastic technique that could affect stability of any loaded drug.

Conclusions:-

We proposed ZNPs as a targeting drug delivery system for cancer treatment. In this study, we evaluated the availability and safety of ZNPs for using in human treatment. The preliminary safety tests showed no acute toxicity to the cells so ZNPs is an excellent safe targeting carrier for drugs to cancerous tissue.

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