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Original Research Paper

Skin histological development in rat embryos exposed to chitosan nanoparticles

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Abstract: Chitosan is a natural nontoxic biopolymer derived by the deacetylation of chitin. Chitosan and its derivatives have attracted considerable interest due to their antimicrobial and antifungal activity.1–3 Chitosan exhibits its antibacterial activity only in an acidic medium because of its poor solubility above pH6.5.4 The antibacterial activity of chitosan is influenced by a number of factors that include the type of chitosan, the degree of chitosan polymerization and some of its other physicochemical properties. The present study was conducted to investigate the damage induced by chitosan nanoparticles at various concentrations on embryonic skin histogenesis . Material and methods: chitosan nanoparticles were synthesized via the ionotropic gelation of chitosan with TPP (sodium tripolyphosphate) anions. . Laboratory pregnant wistar albino rats were injected with different bolus doses of chitposan nanoparticles intraperitoneally and rat pups were dissected after delivery . skin tissues from rats pups were examined histologically and compared to control animals. Results: histological examination of all tissues in both groups shows gradual cytotoxic effect of chitosan nanoparticles reaching extreme histological damage with increased bolus dose. Skin damage included hemorrhaging and distruption of epidermal layers. a decrease in epidermal thickness between the control group and the groups treated with different cyclophosphoamide concentrations Conclusion: This study concludes that chitosan nanoparticles have a strong cytotoxic effect on rat embryonic skin histogensis . Morphological appearance shows variable cell changes to include apoptotic changes as well as nuclear damage. Cytotoxic effect and histological damage is confirmed by the gradual decrease in epidermal thickness of rat pups as well the profound histological changes that is seen in various organs of rat pups.

Keywords: chitosan nanoparticles, epidermis, embryonic, histology.

1.Introduction

Chitosan is a natural polysaccharide derivative of chitin with biodegradable, biocompatible, non-toxic characteristics [1] and it is known to have immunoenhancing effects, antitumor, antifungal and antimicrobial activities. Beside these, it can act as a stabilizing agent and can be used to tailor nanocomposite properties and also to provide long-term stability to the nanoparticles by preventing particle agglomeration [2] developed a new strategy to exploit the use of chitosan as a reducing agent for the production of AuNPs. Interestingly, chitosan-AuNPs were bactericidal against antibiotic resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* [3]. *In vivo* and *in vitro* studies regarding cytotoxicity of nanoparticles have contradictory results and there is still not enough

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information on their effects. In this context, we investigated the cytotoxic effect of chitosan-AuNPs on C26 (murine colon carcinoma) and HeLa (human cervical carcinoma) cell lines. The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task. One approach is the use of colloidal drug carriers that can provide site specific or targeted drug delivery combined with optimal drug release profiles.

The concept of using submicron drug delivery systems for drug targeting was

conceived and developed after Paul Ehrlich originally proposed the idea of tiny drug-loaded magic bullets over a hundred year ago [4]. Among these carriers, liposomes and micro/nanoparticles have been the most extensively investigated. Liposomes present some technological limitations including poor reproducibility and stability, and low drug entrapment efficiency. Nevertheless, several low molecular weight drugs are now commercially available which employ this technology. Polymeric nanoparticles, which possess a better reproducibility and stability profiles than liposomes, have been proposed as alternative drug carriers that overcome many of these problems. Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached.

Nano-sized particles can be administered intravenously because the diameter of the smallest blood capillary is approximately 4 μm. The biodistribution of nanoparticles can vary depending on the size, surface charge and hydrophobicity of the administered particles [5] Particles greater than 100 nm in diameter are rapidly taken up by the reticuloendothelial system (RES) in the liver, spleen, lung and bone marrow, while smaller-sized particles tend to have a prolonged circulation time. Negatively-charged particles are eliminated faster than positively-charged or neutral particles [1]. In general, opsonins (serum proteins that bind to substrates leading to their being taken up by the RES) prefer to adsorb on hydrophobic rather than hydrophilic surfaces. The creation of a hydrophilic coating (such as polyethylene glycol (PEG) or a nonionic surfactant) on hydrophobic carriers significantly improves their circulation time [6]. Together, these data suggest that generating nanoparticles with a hydrophlilic but neutral surface charge is a viable approach to reduce macrophage phagocytosis and thereby improve the therapeutic efficacy of loaded drug particles. The most promising drugs that have been extensively studied for delivery by this route are anticancer agents. Following intravenous injection, many nanoparticle systems including chitosan NP exhibited a marked tendency to accumulate in a number of tumors [6]. One possible reason for the phenomenon may involve the leakiness of tumor vasculature [7]. Doxorubicin loaded chitosan NP showed regression in tumor growth and enhance survival rate of tumor-implanted rats after IV administration. In addition, chitosan NP less Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable, nontoxic, and inexpensive. Furthermore, it possesses positively charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. In the last two decades, chitosan nanoparticles (chitosan NP) have been extensively developed and explored for pharmaceutical applications. Thus, this review focuses on the chitosan nanoparticle preparation technology, applications and mechanism of cell entry.

2.Methodology

Preparation of chitosan nanoparticles : Chitosan was obtained from sigma Aldrich. Chitosan nanoparticles (CHNPs) were synthesized via the ionotropic gelation of chitosan with TPP (sodium tripolyphosphate) anions. The sodium tripolyphosphate solution (1 mg/ml) was prepared by double dis- tilled water. Chitosan nanoparticles were spontaneously fabricated with the drop wise addition of TPP solution (CH/TPP = 4:1) under magnetic stirring (1000 rpm) at room temperature. The suspension was shaped under the similar above mentioned conditions. The nanoparticles were separated by centrifugation at 20,000 g and 14°C for 30 minutes and supernatants was discarded, freeze- dried and stored at 5°C ± 3°C. The weight of freeze-dried nanoparticles were calculated.

SEM (Scanning Electron Microscopy) Analysis

To observe the morphological features, Field Emission Scanning Electron Microscopy, JEOL, JSM-6700F, Japan was used. By using sputter coated with a 5 nm thick

gold, the samples were sprinkled. Magnification was achieved at 25 000 X.

Experimental Animals: fifteen adult female Wistar albino rats weighing between (200-250) g were obtained from the Animal house at the department of biology, university of kufa between the period of December 2021- April 2021. The animals were kept in adjusted laboratory conditions (temperature= 25 ℃, humidity = 60 ± 10%). Animals had free access to standard rat chow and water. Guidelines of the Ethical Committee of the animal Research were followed, which conform to the recommendations on Health Guide for Care and Use of Laboratory Animals. Female rats were allowed to Animals were divided into three groups (control = N5, maternal intraperitoneal injection group (150 mg/kg): N 5 intraperitoneal injection group (300 mg/kg) : N5). Each group was injected daily with allocated dose of cyclophosphoamide intraperitoneally until delivery.

Chitosan nanoparticle dose: Each pregnant animal in each group received 1 ml of specific dose of chitosan nanoparticle dissolved in PBS intraperitoneally while control group received 1mil of PBS (phosphate buffered saline) intraperitoneally. Dose formulation of cyclophosphoamide was achieved following standard protocols and specific doses used in this experiment were 150 mg/kg, 300 mg/kg.

Sacrifice and processing: To carry out a histological analysis, skin, and liver were excised from all liter after birth using the surgical blade and were fixed in 4 % formaldehyde for three days. Tissues were processed and embedded in paraffin wax using TP1020 tissue processor (Leica Microsystem, UK). Five-micron sections were cut using a microtome (Leica Microsystem, UK), which were then placed on slides (Leica Microsystem, UK). The sections were deparaffinised in xylol and then rehydrated by using descending concentrations of alcohol before rehydration in distilled water. Haematoxylin and Eosin were used to stain the sections (Leica Microsystem, UK). Histological investigation of the prepared tissues was carried out by light microscopy with a magnification of 10x. Image J software (NIH, USA) was used to analyse microscopical images to measure the epidermal thickness. The epidermal thickness was measured from the base of the stratum germinativum to the surface of the keratin layer.

Statistical analysis: The mean of the epidermal thickness are measured using image j software. The statistical analysis of the treated groups were compared to the control group. The data were analysed using GraphPad Prism V9.0 software..

3.Results

Scanning electron microscopy examination of chitosan nanoparticles shows small round particles measuring between 40-50 nm at a magnification of 130 000 (figure 1). Chitosan nanoparticles were synthesized from chitin and were produced as a nanoparticles as per scanning electron microscope examination. The measurement of nanoparticles ranged between 40-50nm as shown by many studies who followed the same procedure as shown in this study. study by al howail and colleagues [1] shows significant histological differences in rat liver injected with a weight specific dose of cyclophosphoamide. In their study Hepatdestructive changes were gradually increased from slight hemorrhaging to lymphocytic infiltration indication of cytotoxic effect of cyclophosphoamide injected intraperitoneally. The current study shows similar results to that documented by alhowail and team [1] who demonstrated the profound effect on histomorphological changes in various rat organs. Histological damage present in the current study is concurrent with many scientific evidence which demonstrated the cytotoxic and histological damage caused by cyclophosphoamide injection. However there was lacking scientific evidence present that compares histological changes induced by cyclophosphoamide injected using two different methods intraperitoneally and subcutaneously.

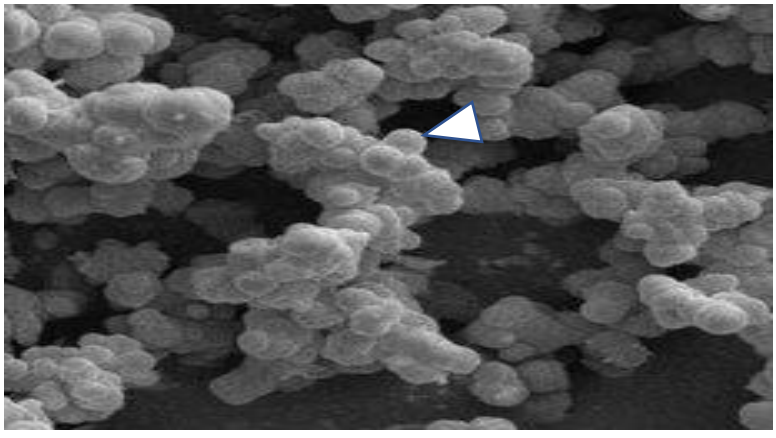

Fig.1. Scanning electron microscope image of chitosan nanoparticles at a magnification 130 K

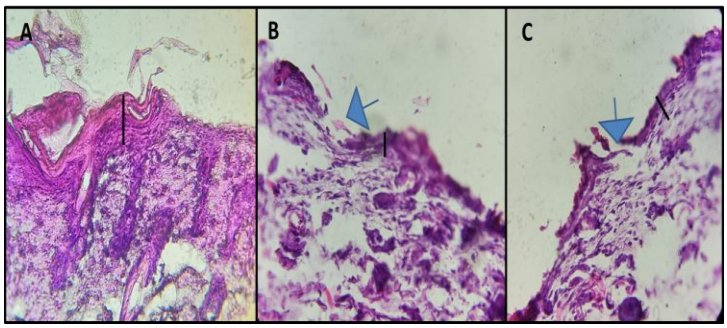

Fig.2. Histological appearance of the skin tissues:

Control group (A) is seen with no epidermal changes. Images (B-C) showed morphological and histological changes including excessive hemorrhaging and sloughing of corneum layer arrows H&E (X400).

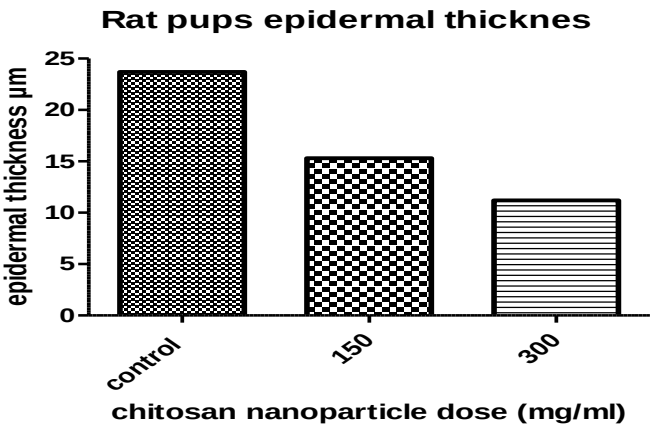

Fig.3. Histomorphometric analysis of the epidermal thickness between the control groups and the groups treated with chitosan nanoparticles. General decrease in epidermal thickness is seen in both groups of rat pups injected with chitosan nanoparticles intraperitoneally. This is also seen in the histological appearance of tissue architecture in figure 2.

chitosan nanoparticles have been synthesized from chitin, a polysaccharide found in the exoskeletons of crustaceans, and have been characterized using scanning electron microscopy (SEM). The nanoparticles have been found to have a size range of 40-50nm, which is consistent with previous studies that have used similar synthesis methods
anoscale materials, particularly gold nanoparticles, have shown promising results in inhibiting bacterial growth. However, the cytotoxicity of gold nanoparticles is still a topic of debate, and further research is needed to fully understand their interactions with mammalian cells. The incorporation of gold nanoparticles into chitosan films has the potential to create innovative nanocomposites with enhanced antibacterial activity and reduced cytotoxicity. Our study demonstrates the importance of considering the physicochemical properties of the polymer and the resulting AuNP characteristics in the development of these nanocomposites. Furthermore, the evaluation of antibacterial activity and cytotoxicity of these nanocomposites is crucial in determining their potential biomedical applications. Overall, the development of novel antimicrobial agents, such as chitosan-gold nanocomposites, is essential in addressing the growing problem of multidrug-resistant microorganisms and improving public health outcomes [3].

conclusion

In summary, chitosan nanoparticles have been successfully synthesized from chitin and characterized using SEM, with a size range of 40-50nm consistent with previous studies. Cyclophosphamide, a chemotherapeutic agent, has been shown to cause hepatotoxicity and histological damage in various organs, including the liver, as demonstrated by the study by Al Howail and colleagues. However, there is a significant knowledge gap in the scientific literature regarding the comparison of histological changes induced by cyclophosphamide injected using different methods, specifically intraperitoneal and subcutaneous injection. Further research is necessary to address this gap and better understand the effects of cyclophosphamide administration on rat organs.

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Author's Contributions

All authors had an equall contribution in the preparation and of the manuscript.

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