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N-Acetylcysteine encapsulated niosomes as antitumor nanoparticles

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Abstract

N-Acetylcysteine (NAC) is an antioxidant compound with antitumor properties. In this study NAC was encapsulated into niosomes for treatment tumor in mice. NAC- encapsulated niosomes' morphology and Fourier-transform infrared spectroscopy (FTIR) analysis were measured. Fifteen mice male BALB/c were divided to three groups: group1: negative control, group2 positive control (tumor group), group3 treated with NAC- encapsulated niosomes after tumor growth. The effect of NAC- encapsulated niosomes on the tumor was determined by measuring tumor size progress, comet assay, oxidative stress parameters (GSH, Nitric Oxide, histopathological investigation.Transmission electron microscopy examination found that NAC- encapsulated niosomes have spherical shape. Tissues histological studies emphasized the protective effect of NAC- encapsulated niosomes against tumor. In conclusion oral delivery of NAC- encapsulated niosomes improved antitumor effect.

Keywords: Niosomes; N-Acetylcysteine; Tumor; Histopathology.

1. Introduction

Nanotechnology is a new branch of science that deals with the creation and development of nanomaterials [1,2]. In 1909, Paul Ehrlich envisioned a medicine delivery technique that would directly target sick cells, starting off the development of targeted delivery. Drug targeting is the capacity to focus a medicinal drug to a specific region of action with minimal or no interaction with non-target tissue [3,4]. Latest advances in nanotechnology are paving the way for the creation of nanomedicine agents, which have huge potential for improving cancer treatment [5,6].

Nowadays nanoparticles have been studied for a variety of therapeutic uses, including medication transporters, gene transfer to malignancies, and imaging contrast agents [7]. They have received a lot of attention in the drug delivery system for cancer therapy due to their offered optimal size and surface features capable of increasing therapeutic efficacy by improving hydrophobic drug solubility and extending drug half-life [8-11]. Therefore, nanostructured materials can passively accumulate at tumor sites due to their increased permeability and preventing drug resistance in cancer cells [12-14]. For example, liposomes and niosomes, are colloidal drug delivery vehicles that offer specific benefits over traditional dosage forms [15]. These systems can serve as medication reservoirs, releasing the active ingredient in a regulated manner. Furthermore, altering their composition or surface can enable targeting [16].

Niosomes have a bilayer structure and are produced in an aqueous phase by combining nonionic surfactants with cholesterol [17,18]. They have solved some of the drawbacks of liposome drug carriers, such as chemical instability, phospholipid purity variability and expensive costs [19,20].

NAC is the most prevalent component in the garlic extract [21]. It has been commercially available for a long time as a safe and affordable medicine [22]. NAC with a molecular formula C5H9NO3S and molecular weight 163.19 Da has a solubility of >24.5 g/mL in water and it is resistant to extreme lighting conditions and temperatures above 120oC. It has

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significant antioxidant properties both intracellularly and extracellularly due to its biochemical features [23,24].

Ehrlich's tumor has long been employed in experimental oncology to test the therapeutic potential of various synthetic chemotherapeutic drugs or to assess the anticancer activity of various natural compounds [25].

In this study, a thin film layer hydration method was used to prepare niosomes as a carrier of N-acetyl -l-cysteine for cancer treatment. After synthesis of NAC-loaded niosomes, we investigated the effect of an oral dose of the formulation on Ehrilch tumor in mice. The niosomes were characterized using different techniques as transmission electron microscope (TEM) and fourier-transform infrared spectroscopy (FTIR). In-vivo studies were also performed using a mice-based model.

2. Experimental

Materials

N-Acetyl-L-cysteine (MW: 163.19 g/mol, Melt. Point: 106-108 0C (intoxicated), powder), tween and cholesterol were purchased from sigma Aldrich. Phosphate buffer saline tablets (PBS) pH 7.4 (purity ≥ 99%) was purchased from Bio Shop Canada Inc. Ethanol was purchased from Fisher Scientific UK.

Preparation of niosomes encapsulating NAC

In a round bottom flask, Tween and cholesterol (1:2) were dissolved in ethanol using the film hydration technique [26]. The solvent was evaporated using a rotary device (Janke & Kunkel RV05-ST, Germany) under normal circumstances (50 rpm and 45°C). After the solvent was removed, a thin and uniform layer developed on the flask's wall [27,28]. The film was hydrated with PBS buffer PH 7.4 containing NAC. The final solution was sonicated (ultrasonic bath, Daihan, made in Korea) for five minutes to enhance the development of regular shaped niosomes and inhibits their aggregation [27]. Niosomes were centrifuged at 10,000 rpm for 30 minutes at 4°C in order to isolate unloaded drug. The precipitate was twice rinsed in buffer. Using the same method, free niosomes were prepared without loading drug [29].

Characterization of niosomes

Transmission electron microscopy (TEM) (FEI Tecnai G20, Super twin, Double tilt, LaB6 Gun) operating at 200 kV, was used to study the particle sizes and morphology of niosomes.

Fourier transforms infrared (FTIR) (Thermo Nicolet, AVATAR, 370 FT-IR, USA) spectra were obtained to evaluate the influence of NAC on niosome. The freeze dried samples were combined and crushed with KBr pellets to make a tablet then the scans were performed between 4000 and 400 cm^{-1} [30].

Experimental design

Adult male BALB/c mice of average weight 23 g, 8-10 weeks old, were injected subcutaneously with Ehrlich carcinoma cells (obtained from the National Cancer Institute “NCI”, Cairo University) in their right thigh, where the tumor grew in a single and solid form [31]. All animal treatments and care were performed based on the guidelines for the Care and Use of Laboratory Animals, Cairo University Institutional Animal Care and Use Committee (CU-IACUC), based on reviewing the application number CU/I/F/84/19. Animals were given an oral solution of NAC loaded niosomes through orogastric gavage daily for two weeks as part of the treatment protocol to test if NAC provided as a niosomal formulation was beneficial in reducing tumor growth [32,33].

A total of 15 mice were used and were randomly divided into three groups, each containing five mice, Group 1 was negative control group (with saline), Group 2 was the positive control group (with tumor), Group 3 received NAC loaded niosomes after tumor growth . At the end of the treatment, all of the mice were sacrificed; tumor tissues were rapidly removed, washed with isotonic saline, split into sections, and utilized for evaluation.

Histopathology

Tumor tissues (n=3 per group) were immersed in a 10% neutral buffered formalin solution, then dehydrated in alcohols, cleaned in xylene, sectioned, stained with Haematoxylin and eosin and finally embedded in paraffin blocks. The slices of the tissue were examined using an optical microscope (Olympus CX31 microscope, Tokyo, Japan) linked to a digital camera (Canon) [34-36].

Statistical analysis

The data was analyzed by SPSS v. 16.0 for Windows. The significant differences were calculated using one-way analysis of variance (one-way ANOVA). $P \leq 0.05$ was judged significant.

3. Results and Discussion

Characterization of niosomes

The surface morphology evaluation by TEM (fig.1) confirmed that NAC loaded niosomes exhibited a spherical shape with a homogenous distribution and the particle size of the prepared niosomes [37].

Infrared spectroscopy is one of the most crucial investigations to identify the functional groups, determine potential interactions between the chemicals, and determine whether the drug was contained within niosomes [38]. In the spectrum of NAC (fig.2a), the peak found around 3100-3400 cm^{-1} can be assigned to hydrogen bonded stretch (–O-H) of the carboxylic group present in NAC and in the FTIR spectrum of the empty niosomes samples (Fig 2 b), The peak around 1000-1100 cm^{-1} is assigned to C–O stretch in ether and ester groups. The peak around 1200-1700 cm^{-1} demonstrated the occurrence of C=O prolong to the ester group, and –CH2 bending in lipids and surfactant, respectively. Around 2900–3000 cm^{-1}, -CH3 asymmetric and symmetric stretching was observed. In the spectrum of NAC loaded niosomes (fig.2c), there were no additional peaks appeared compared to the spectrum of empty niosomes, which confirms the encapsulation of NAC inside niosomes.

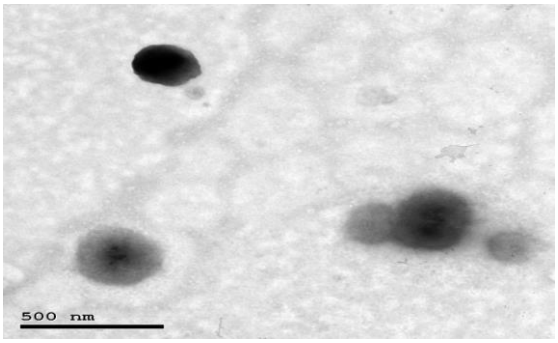

Fig. 1. TEM image of NAC loaded niosomes.

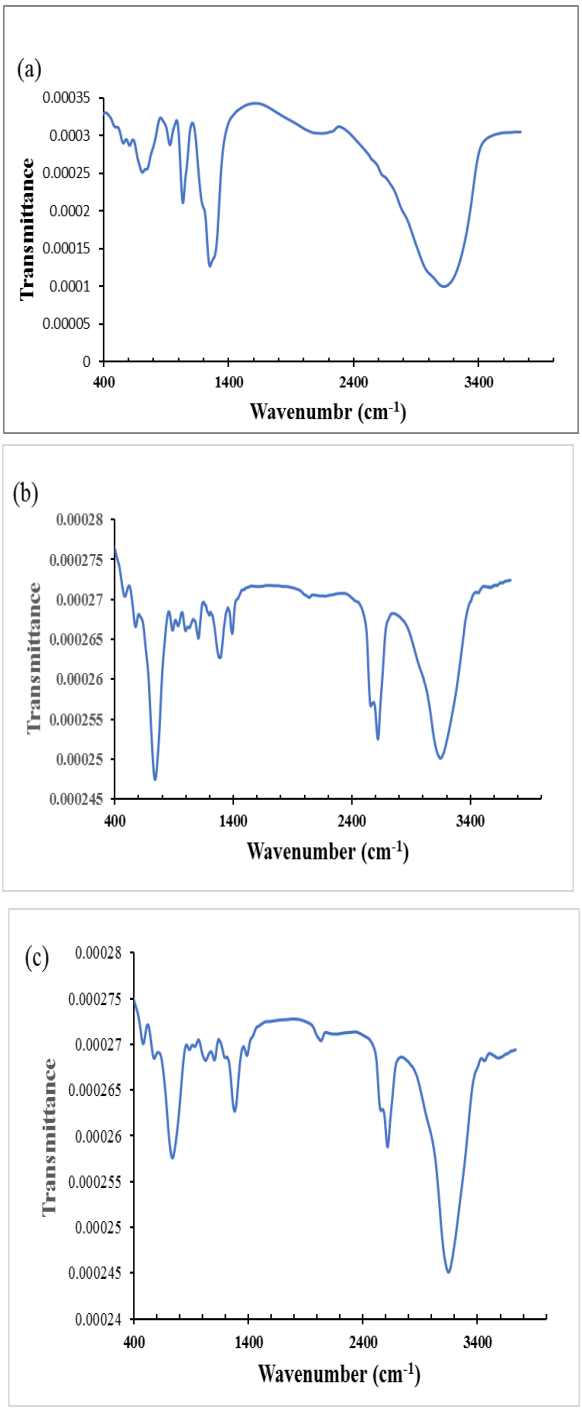

Fig. 2. FTIR spectrum of a) NAC , b) NIOs and c) NAC loaded niosomes.

Histopathology

According to histological examination (fig.3), numerous big, irregular vessels were seen in the tumor group and after treatment with NAC loaded niosomes , the striated skeletal muscles deteriorated, with considerable infiltration of mononuclear inflammatory cells.

The obtained data indicated that NAC-coated niosomes have great potential for treating cancer cells as the nanoparticle shapes enhance their power to penetrate deeply into cells while enhancing their concentration which enhances their antioxidant capacity.

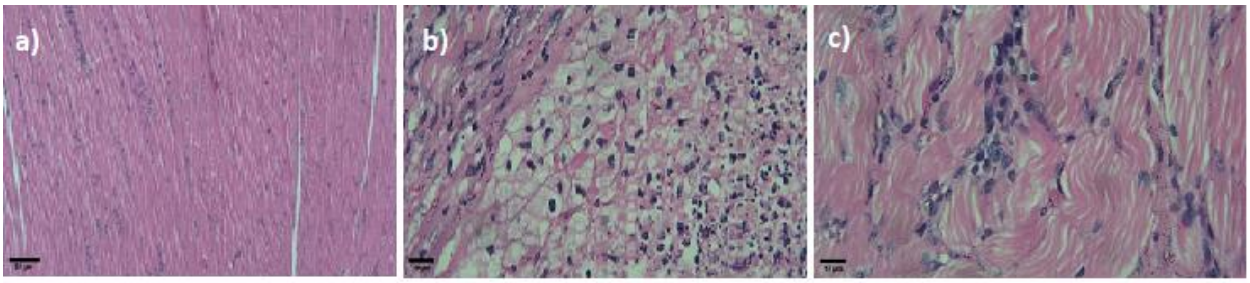

Fig. 2. Histopathology photomicrographs in muscle tissue of group 1 (a) Normal striated skeletal muscles (H&E, X400) , group 2 (b) Degenerated striated skeletal muscles with coagulative necrosis (nuclear dust and/or totally pyknotic nuclei) and moderate mononuclear inflammatory cells infiltration were seen. Irregular bluish purple deposits of calcium in damaged tissues (calcification) were noticed (H&E, X400). , group 3 (c) Degenerated striated skeletal muscles with moderate mononuclear inflammatory cells infiltration were seen (H&E, X400).

4. Conclusions

In conclusion, the findings of this study demonstrate that NAC loaded niosomes inhibits tumor growth, As a result, new cancer therapy techniques might be devised, such as loading NAC on niosomes.

Conflicts of interest

"There are no conflicts to declare".

Acknowledgments

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