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Antitumor efficacy of Curcumin nanoparticles

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Abstract

The present study tried to evaluate the anticancer ability of curcumin by decreasing its adverse effects and increasing its bioavailability. In this work curcumin was loaded in nano emulsions then characterized by transmission electron microscope (TEM). The antitumor efficacy of the curcumin nano emulsions was assessed in mice by DNA comet assay analysis, FTIR and examination of tumor histopathology. The obtained results found that mice treated with curcumin nano emulsions were effective in reducing DNA damage and changes in tumor tissue structure. In conclusion, curcumin nano emulsions have potential efficiency in treating tumor tissues.

Keywords: Nano-curcumin; Tumor; Comet.

1. Introduction

Cancer is one of the serious health problems. Cancer are cells having abnormally invasion, spread and metastasis [1 and 2] and it is one of the most common causes of death among humans. While treatment regimens such as chemotherapy and radiotherapy have negative health impact, there are intense efforts to find new methods. Researches aim to find anti-proliferative medications with less side effects on the immune system [3].

Phytochemicals have antitumor and anti-inflammatory properties, and have the least side effects on normal cells [4].

Curcumin is a natural lipophilic polyphenol compound which has promising antitumor properties, this is due to its mechanisms of action. [5-8]. Curcumin has antitumor activity that can interrupt the cell growth and rising the apoptosis by increasing reactive oxygen species generation, interacting with enzymes leading to modulation of different signaling pathways [9- 11].

Unfortunately, curcumin's poor bioavailability, rapid excretion and low solubility in water limited its benefit effect [12]. Therefore many different technological methods were used to improve its quality such as encapsulating of curcumin in sodium alginate/ZnO hydrogel [13], using chitosan and tripolyphosphate with different concentrations to form curcumin nanoparticles [14], producing iron oxide nanoparticles coated with curcumin [15].

In this study, nano-emulsions encapsulating curcumin were prepared and then characterized by TEM. The antitumor effect was investigated in mice.

2. Experimental

Materials

Curcumin , Span® 20 (sorbitan laurate), lauric acid, isopropyl myristate and Tween® 80 (polysorbate 80) were obtained from Sigma-Aldrich. Phosphate buffer (PBS) was purchased from MP Biomedical, LLC (USA).

Curcumin, Lauric acid, sorbitan laurate, polysorbate 80, 2-phenyl benzimidazole 5-salfonic acid(pbsa) and isopropyl myristate were obtained from Sigma-Aldrich (Germany). Phosphate buffer (PBS) from MP Biomedicals, LLC (USA).

Preparation of nano emulsions encapsulating curcumin

Nano emulsion encapsulating curcumin was prepared. Span® 20 (0.16 ml), isopropyl myristate (0.6 ml), Tween® 80 (3 ml) and lauric acid (100 mg) were mixed, then curcumin (125 mg) was introduced to the solution and stirred on a magnetic stirrer (MS-300HS, Misung Scientific, Gyeonggi-do, Korea) at room temperature. A 8 ml distilled water was added to the solution drop by drop. Curcumin nano-emulsions were formed and kept in the refrigerator for 24 h to equilibrate [16-19].

Curcumin nano emulsions characterization

Transmission Electron Microscope (TEM, FEI Tecnai G20, Super twin, Double tilt, LaB6 Gun) was used to determine the size and morphology of curcumin nano emulsions.

Animals experiment

The animal experimental protocols were carried out in accordance with the Guide for the Care and

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Use of Laboratory Animals and Use Committee (CU-IACUC), the approval number is CU/I/F/1/21. Albino male mice (~25 g), of age 8–10 weeks, were obtained from the National Cancer Institute "NCI", Cairo University, Giza, Egypt. Ehrlich ascites carcinoma (EAC) was purchased from the Tumor Biology Department, National Cancer Institute "NCI", Cairo University. Under sterile conditions, Ehrlich's ascites carcinoma cells were prepared and 0.1 ml (2.5 × 106) of the tumor cells was injected in the right hind limb of each mouse to induce solid tumor. In all injected animals, tumor developed in 11 - 14 days post tumor induction.

Fifteen mice were randomly divided into three groups (n=5): control, tumor and curcumin nano emulsions group. The control and tumor groups received PBS orally, and the curcumin nano emulsions group received 20 mg/kg curcumin nano emulsions daily for 2 weeks [20]. At the end of the experiment, mice were sacrificed; Tumor tissue was removed, cleaned with saline and used for evaluation.

Fourier-transform infrared spectroscopy (FTIR)

The tumor tissue samples were lyophilized then assessed by FTIR. Tissues were mixed with KBr at a 1:100 ratio and are pressurized with a hydraulic press. The pellets of each sample was scanned over the range 400–4000 cm^{-1} by type A FT/IR-4100 (A Basic Vector, Germany) [21].

Comet assay

Comet assay is a visible and sensitive way used to assess DNA damage [22]. DNA damage that created in tumor tissue of all experimental groups' was estimated using this technique [23]. comets were observed by fluorescent microscope with a magnification power x 400. DNA length migration, DNA percentage migrated and the olive moment were measured by Kinetic Imaging, Ltd. (Liverpool, UK) created Comet 5 image analysis software, which is connected to a CCD camera [24,25].

Histological examination

Tumor tissue samples were inserted in 10% buffered formalin solution then fixed into paraffin bars and sectioned in 5 μ thick slices. The slices were stained with hematoxylin and eosin [26]. The tissue slices examination was carried by optical microscope (CX31 Olympus microscope, Tokyo, Japan) linked with a digital camera (Canon).

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

The present work formulated and characterized curcumin nano emulsions using TEM technique to examine its morphology and size (Fig. 1). TEM image shows a non-aggregated, round-shaped and well-dispersed nanoparticles having a diameter between ~20 nm, restricts that it is very small in size and can enter into cells without causing aggregation.

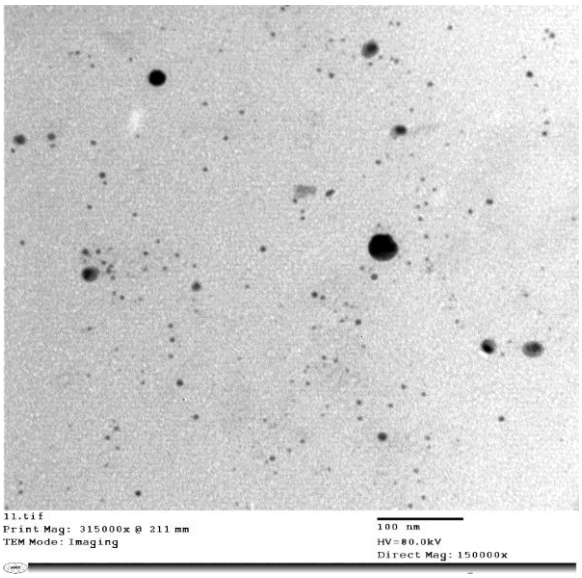

Fig. 1. TEM image of curcumin nano emulsions.

FTIR spectroscopy, comet assay and histopathology were used to assess curcumin nano emulsions therapeutic efficacy in mice bearing solid tumor. Figure 2 showed the normalized FTIR spectra of control, tumor, and tissue samples. FTIR spectra provide good information at the molecular level. Significant differences between normal and tumor tissues were seen in FT-IR spectra in this study. It could be caused by the changes of protein, fat, sugar and nuclear acid in cells. It turns out that the tumor sample generated different spectra for in comparison to both control and curcumin nano emulsions treated samples. The amide II (1530 –1580 cm − 1) and amide I (1610 –1730 cm − 1) bands have the most affected changes due to their information's related to the protein structure, which were confirmed by data in [27,28].

The amide II and amide I bands were weak and shifted to the left and changed in tumor tissue intensities comparing to normal tissues and tissues that treated with curcumin nano emulsions.

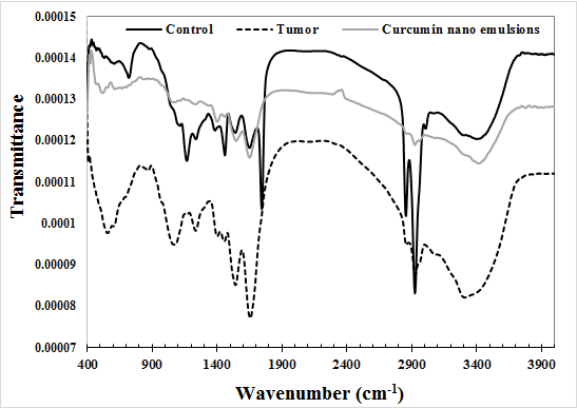

Fig. 2. FTIR of control, tumor and curcumin nano emulsions tissues.

Moreover, modulation tumor effect is reflected in DNA damage. Comet assay for normal tissues, tumor tissues and that treated with curcumin nano emulsions were measured. The % DNA in tail, olive tail moment and tail length parameters were calculated (Fig. 3 a,b) [29,30]. The curcumin nano emulsions treated mice group showed a significant decrease comet parameter comparing to tumor group. These data were supported by the comet images in Figure 3. This treatment effect may be result from the high penetration ability of curcumin nano emulsions. The emulsion can penetrate the nucleus and mitochondria membrane then help in decreasing the oxidative stress [31].

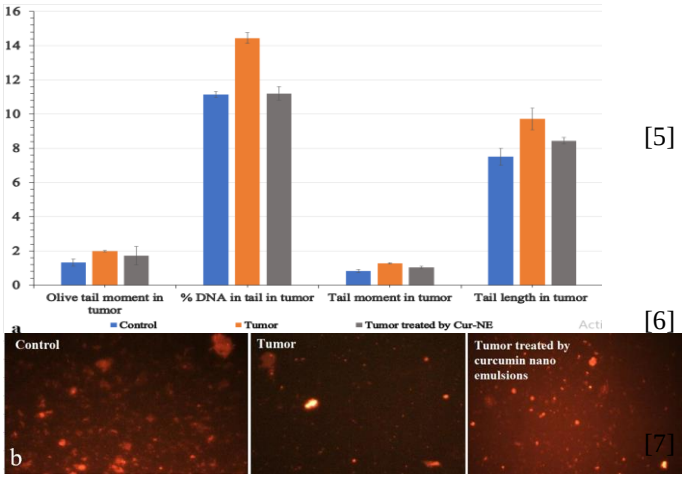

Figure 3. a) Comet parameters % DNA in tail, Tail length, Tail moment, Olive tail moment, b) Comet images of control, tumor and tumor treated by curcumin nano emulsions.

Figure 4 shows the histopathological changes in normal, tumor and curcumin nano emulsions tissues. The tumor cells show hyperchromatism and mitotic activity compared to normal muscles cells. Moreover, the tissue sample from mice group treated with curcumin nano emulsions showed inhibition of tumor cells.

4. Conclusions

In this study curcumin nano emulsions were prepared and described by TEM. It showed an effective property against tumor cells. Their effect may be by inhibition of the progress of the tumor cells through pushing large amounts of curcumin into cells and then curcumin reacts with free radicals.

5. Conflicts of interest

"There are no conflicts to declare".

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