CONSORTIUM

Original Research Article

NOVEL OLEUROPEIN NANOCAPSULAR FORMULATION: PREPARATION, CHARACTERIZATION AND ANTICOLON CANCER ACTIVITY

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ABSTRACT

Nanotechnology has provided substantial contribution in the field of nutraceuticals' delivery. In the current work, a novel nanocapsular system loading the nutraceutical oleuropein was prepared and characterized, then further tested for its efficacy against colon cancer cells. Oleuropein nanocapsular formulation was prepared by the phase inversion method. Oleuropein nanocapsules were characterized for their particle size, zeta potential, polydispersity index, morphology, *in vitro* drug release, stability and anticancer activity on HCT-116 colon cancer cells. Oleuropein nanocapsules were successfully prepared with a particle size of 151 nm, polydispersity index of 0.286 and an overall neutral charge. Moreover, it was able to sustain the release of drug for 24 hours with a cumulative percent released of 100%, compared to oleuropein control which displayed 100% release after only 2 hours. Transmission electron microscopic examination of the nanocapsules displayed their small particle size and revealed their spherical morphology in the form of core and coat. The prepared nanocapsular formulation was stable, with no significant changes in particle size, polydispersity or charge observed upon storage. Interestingly, the oleuropein nanocapsular formulation was 28 times more cytotoxic than oleuropein alone on HCT-116 colon cancer cells. Therefore, it can be delineated that nanocapsules act as promising delivery carrier for oleuropein, which is worthy of further experimentation *in vivo* to determine its bioavailability.

Keywords: Oleuropein, nanocapsules, colon cancer, HCT-116 cells.

INTRODUCTION

Nanotechnology was proven to enhance the therapeutic effectiveness of many drugs in the treatment of different diseases, among which is cancer [1]; since they allow either passive or active targeting to the tumor tissues. Recently, antioxidant nutraceuticals were reported as promising molecules for treatment of cancer, owing to their efficacy and considerable safety. Among the promising nutraceuticals in this regard is oleuropein, which is a bioactive phenolic compound present in the leaves of olive tree [2]. It was reported to be effective against several types of cancer [3], among which is colon cancer. Its exact mechanism of anticancer activity in colorectal cancers was verified in HT-29 cell lines, in which it was reported to activate p53 pathway which causes cellular apoptosis [4].

Inspite of the effectiveness of oleuropein, it was reported to be exhibit poor oral absorption

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owing to its high polarity [5]. Therefore, it can be hypothesized that the incorporation of oleuropein in a surfactant-based nanoparticulate system might be a feasible means for enhancing its delivery and increasing its anticancer activity in colon cancer. Nanoparticles were reported to potentiate the anticancer activity of drugs [6-8], owing to their enhanced internalization within cancer cells because of their unique small size [9]. Among the promising and recently reported nanoparticles are nanocapsules, which are mainly composed of an oily core and a polymeric or surfactant based shell [10]. Lipidic nanocapsules possess a lipoprotein-like composition, and were reported to enhance the anticancer activity of cytotoxic drugs, with reduced side effects [11]. Therefore in the current work, oleuropein was encapsulated in surfactant-based nanocapsules and characterized for its physicochemical properties, and was further tested for its cytotoxic effect against HCT-166 colon cancer cells. To authors' knowledge, only one published paper attempted the encapsulation of oleuropein in nanoparticles, and no papers reported the anticancer activity of oleuropein against HCT-116 cancer cell line.

MATERIALS AND METHODS

Materials

Soybean lecithin (Epikuron 200) was kindly obtained from Cargill Co., Germany. Labrafac Lipophile was kindly obtained from Gattefosse' Co., France. Solutol HS15, disodium hydrogen phosphate, potassium dihydrogen phosphate, dimethylsulfoxide, MTT dye and dialysis membrane (molecular weight cut off 12000-14000) were purchased from Sigma Aldrich Co., USA. Oleuropein was purchased from Skin Actives company, USA. Fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% trypsin-EDTA were purchased from Lonza, Belgium. HCT-116 cancer cells were obtained from the American type culture collection (ATCC, USA).

Preparation of oleuropein nanocapsules

The oleuropein nanocapsules were prepared by the phase inversion method [12]. Briefly, 20 mg of oleuropein and 100 mg soybean lecithin were dispersed in 2.5 gram labrafac lipophile oil by magnetic stirring for 10 minutes, followed by addition of 2.5 gram solutol HS15 and 3 gram distilled water. Magnetic stirring was continued for 5 minutes followed by heating of the dispersion during mixing till the temperature reaches 85°C for the formation of a water in oil emulsion. Cooling of the emulsion was then performed to a temperature of 55°C, in which the emulsion was inverted to oil in water type. The cycle was repeated two more times, followed by addition of distilled water at 4°C till a total weight of 10 grams.

Determination of the particle size, polydispersity index and zeta potential of oleuropein nanocapsules

The particle size, zeta potential and polydispersity index (PDI) of the prepared oleuropein nanocapsules were measured using the Zetasizer device (model ZS3600, Malvern, UK).

Morphological examination using transmission electron microscopy

The prepared oleuropein nanocapsules was examined for its morphology using transmission electron microscopy without staining, after drying on a carbon grid (VERSA 3D, USA).

In vitro release of oleuropein from the prepared nanocapsules

The release of oleuropein from the nanocapsules was performed using a dialysis-based method [13-15], and compared to the release of oleuropein from solution control. One ml of either the drug-loaded nanocapsules or the drug solution was placed in a plastic cylinder of 8 cm height, attached to the shaft of USP dissolution device (Pharma Test, Germany) rotating at 50 rpm and temperature of 37±0.5°C. The release medium was 200 ml phosphate buffer of pH 7.4 containing 2% tween 20, from which three ml samples were drawn at definite time intervals (0.25, 0.5, 1, 2, 3, 4, 6, 24 hours). Finally, the amount of oleuropein released was measured spectrophotometrically at wavelength of 288 nm (SPUV UV/VIS double beam spectrophotometer, SCO TECH, Germany)

Assessment of the storage stability of oleuropein nanocapsules

The physicochemical properties of the oleuropein nanocapsules (particle size, PDI and zeta potential) were measured after 3 months storage at refrigeration temperature, to assess the stability of the prepared formulation [6,16].

Evaluation of the cytotoxicity of oleuropein and its nanocapsules against HCT-116 cancer cell line

HCT-116 cells were grown on RPMI-1640 medium containing 10% inactivated fetal calf serum and 50 µg/ml gentamycin at 37ºC in a humidified atmosphere with 5% CO2, and were sub-cultured two to three times weekly. Cells were then placed in culture medium at a concentration of 50,000 cells per well (Corning® 96-well tissue culture plates) then incubated for twenty four hours. Either oleuropein or oleuropein nanocapsules were added to the cells at different concentrations (0.5-500 µg/ml). The viability percentage values were calculated after 24 hours incubation period, in which the media was replaced with another fresh media containing MTT dye followed by incubation for 4 hours and further addition of dimethyl sulfoxide. The optical density was measured at 590 nm with a microplate reader (SunRise, TECAN Inc, USA) to calculate cell viability according to the following equation [8, 17]:

$$\text{Viability \%} = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ control\ cells} \text{ X } 100\%$$

Statistical analysis

Measurements were done in triplicate and reported as mean±S.D. Student T-test was performed using Graphpad® Instat software, at significance of P≤0.05. The IC_{50} values were calculated using Graphpad Prism software (San Diego, CA. USA)

RESULTS AND DISCUSSION

Measurement of particle size, PDI and zeta potential of oleuropein nanocapsules

Oleuropein nanocapsules displayed a particle size of 151.45±9.97 nm, a PDI value of 0.286±0.08 and a zeta potential value of -0.059±0.004 mV. The small particle size of the nanocapsules is expected to provide better tumor uptake by the enhanced permeation and retention EPR effect [9]. The low polydispersity index value of the nanocapsules (less than

0.4) indicates the presence of a homogenous population of nanocapsules, and the effective solubilization of oleuropein within the nanocapsules. The almost neutral charge of the prepared nanocapsules is caused by the non-ionic nature of the utilized surfactant Solutol HTS15.

Morphological examination of oleuropein nanocapsules using transmission electron microscopy

As shown in Figure 1, the oleuropein nanocapsules displayed homogenous non-aggregated spherical droplets, displaying an oily core and a surfactant shell. The particle size obtained concurred with the particle size obtained with the Zetasizer.

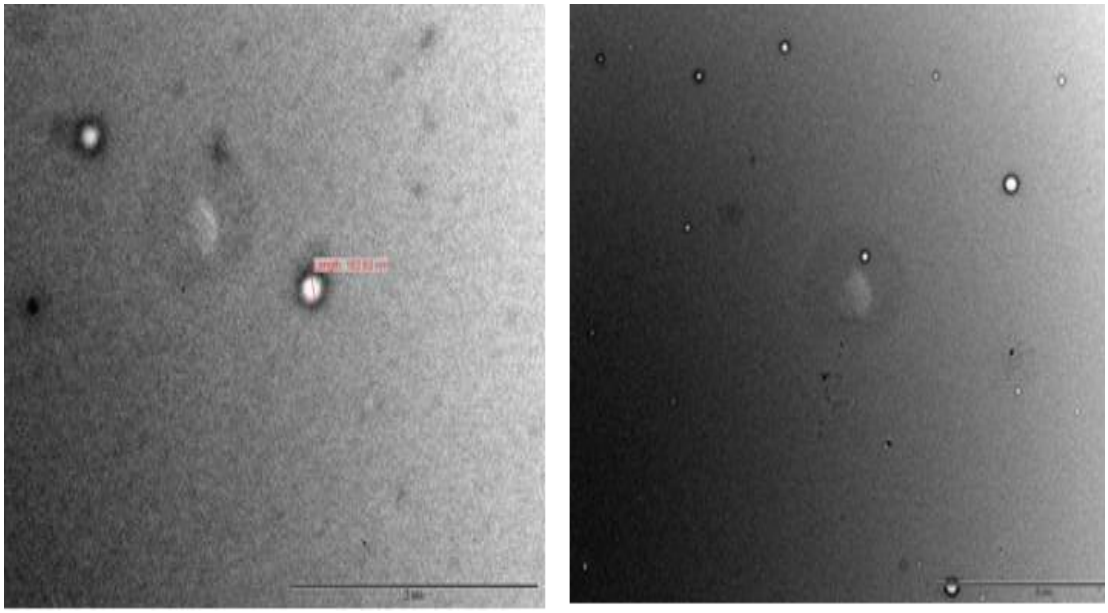

Figure 1. Transmission electron microscopy pictures of the prepared oleuropein nanocapsules.

***In vitro* release of oleuropein from the nanocapsules**

Oleuropein showed a sustained release pattern over 24 hours from the nanocapsules, reaching complete release (100%) as displayed in Figure 2. Meanwhile, the release from oleuropein solution was complete after only 2 hours. The sustained release of oleuropein from nanocapsules is ascribed to the consequent partitioning of oleuropein from the oily phase of nanocapsules into the aqueous medium, and the considerable viscosity of the formulation, creating hindrance for oleuropein release.

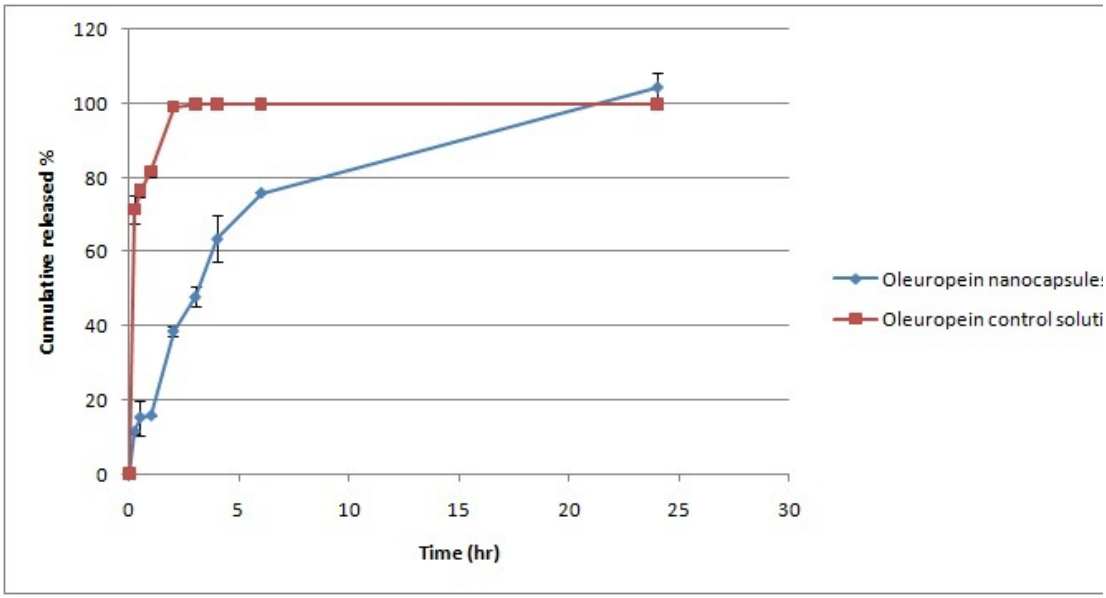

Figure 2. Cumulative percent released of oleuropein for 24 hours from the nanocapsules compared to oleuropein solution.

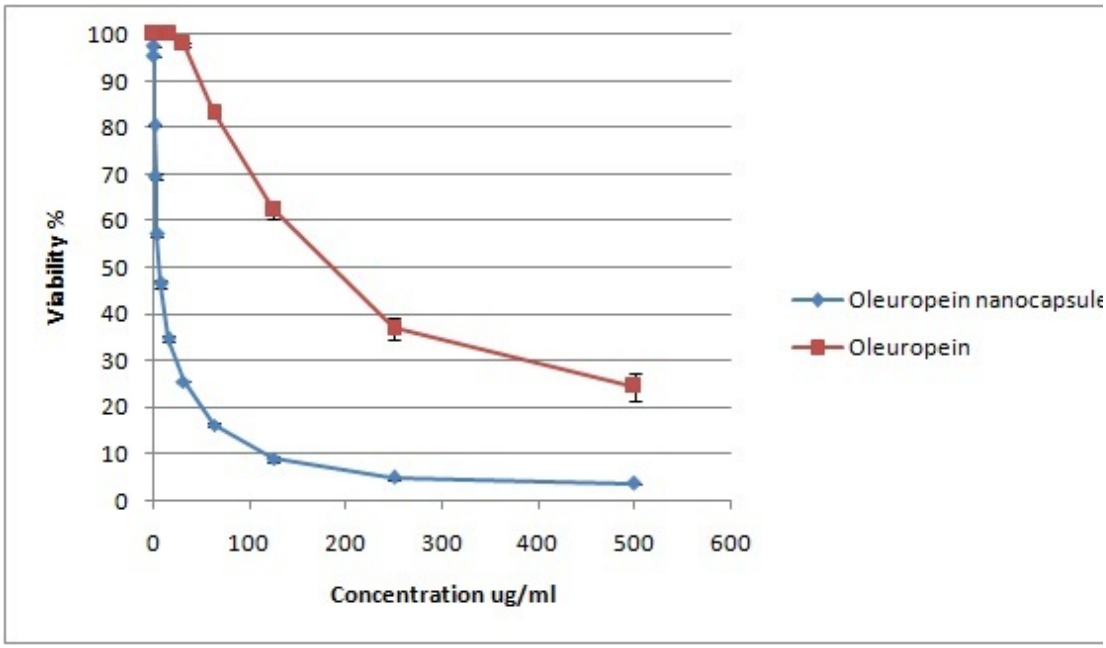

Figure 3. The viability percentage of HCT-116 colon cancer cells as a function of oleuropein concentration.

Stability of oleuropein nanocapsules

After three months storage of the nanocapsules at refrigeration temperature, oleuropein nanocapsules displayed a particle size of 153.85±33.87, a PDI value of 0.28±0.04 and a

zeta potential value of -0.031±0.002. It was found that these values were statistically insignificant compared to the values obtained for the freshly prepared nanocapsules (P>0.05), suggesting the stability of the nanocapsules.

Evaluation of cytotoxicity of oleuropein nanocapsules in HCT-116 cancer cell line

Till current date, no studies were published on the cytotoxicity of oleuropein in HCT-116 cells. The cytotoxicity of oleuropein compared to oleuropein nanocapsules was assessed and displayed in Figure 3. As observed in the previous figure, oleuropein exhibited an IC_{50} value of 185±8.7 µg/ml when administered as free drug, while displaying an IC_{50} value of 6.52±0.7 µg/ml in the nanocapsules form. Oleuropein was about 28 times more potent as an anticancer with significant decrease ($P<0.05$) in the cellular viability when encapsulated in nanocapsules. This could be attributed to the small size of the nanocapsules and their surfactant-based shell, which allows better cellular uptake, and hence enhanced anticancer activity. The reported IC_{50} value for oleuropein in HT-29 cancer cells ranged from 108-216 µg/ml [4], which concurred with the value obtained in this work. The aforementioned results delineate nanocapsules as promising carrier for oleuropein.

CONCLUSION

Oleuropein nanocapsules were proven to be provide high loading of the drug, a sustained release nature in addition to exhibiting physicochemical stability. They were also successful in enhancing its anticancer activity, suggesting that they can be pharmacologically tested in animal models as a futuristic step.

Declaration of interest

The authors report no conflict of interest

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