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Enhanced therapeutic benefit of quercetin-loaded phytosome nanoparticles in ovariectomized rats

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ABSTRACT

Quercetin, a dietary flavonol phytoestrogen, has many health benefits but it is poorly absorbed when administered orally. To improve its bioavailability, we prepared quercetin-loaded phytosome nanoparticles (QP) using the thin film hydration method. The prepared nano-formulations were characterized using different techniques. Transmission electron microscopy revealed the homogeneously spherical, well and uniformly dispersed, nano-sized nature of QP. Dynamic light scattering measurements of QP (70 ± 7.44 nm) also confirmed this. Stability of the formed nanoparticles was established via zeta potential determination. The prepared QP exhibited very high encapsulation efficiency (98.4%). The estrogenic activity of QP, concerning inflammation, oxidative stress, bone, lipid profile, blood glucose level and weight gain, was investigated in ovariectomized rat model using 10 and 50 mg/kg/day oral doses for 4 weeks. Treatment with QP showed significant increase in serum calcium, inorganic phosphorus and glutathione content. Whereas, it significantly decreased serum alkaline phosphatase, acid phosphatase, malondialdehyde level, tumor necrosis factor-alpha and glucose level and improved lipid profile. Consequently, the results obtained confirm the superiority of QP over free quercetin at the same doses as a promising hormone replacement therapy.

1. Introduction

Menopause occurs when women are between 45 and 55 years of age and is associated with different symptoms such as increased food intake and body weight, metabolic dysfunction, loss of bone mineral density, diabetes, impairment of muscle function, hyperlipidemia, psychological and mood changes, increased inflammatory markers and oxidative stress which may result in lipid peroxidation of cell membranes and damage to proteins and DNA [1]. Hormone replacement therapy (HRT) has been chosen as the standard approach to alleviate menopause-associated symptoms (ovariectomy model in animals). However, because of the possible negative effects associated with long-term HRT, especially the increased risk of thromboembolic accidents, stroke and breast cancer, HRT has lost ground among women and a growing interest in alternative strategies has been established. In this regard, there is a particular attention in validating the estrogenic effects of natural herbs with antioxidant/anti-inflammatory potential as well as few or even no significant side effects [2].

Phytoestrogens have been suggested to be selective estrogen receptor modulators and they may be used in postmenopausal women for helping to reduce some health risks associated with lack of estrogen [3]. Quercetin, as a phytoestrogen, has also been found to stimulate both estrogen receptors (ERα and ERβ) with a higher capacity for stimulating ERβ [4]. However, there are inconsistent results in literature regarding the effects of quercetin on ERs as agonistic or antagonistic. Initial studies on the estrogenic activity of quercetin have reported only anti-estrogenic effects on an

Abbreviations: Q, quercetin; QP, quercetin loaded phytosome; EB, estradiol benzoate; FL, free liposome; TEM, Transmission Electron Microscopy; PDI, polydispersity index; DPPH, Diphenyl picryl hydrazine; OVX, ovariectomized; NF-κB, nuclear factor- κB; DLS, dynamic light scattering; ACP, acid phosphatase; ALP, alkaline phosphatase; MDA, malondialdehyde.

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estrogen-sensitive breast cancer cell line (MCF-7) [5], while subsequent studies have found that quercetin exerts both estrogenic and anti-estrogenic effects in a dose-dependent manner [6] and [7].

Nevertheless, phytoconstituents are limited in their effectiveness because they are poorly absorbed when taken orally or even when applied topically. Hence, many approaches have been developed to improve the oral bioavailability to fully utilize the potential of quercetin. Phytosome fabrication provides a unique way to enhance levels of quercetin in plasma for prolonged period, thus enhancing the rate and/or extent of quercetin absorption [8]. There are several reports on quercetin actions as hepatoprotective [9], antioxidant [10], anti-inflammatory [11], gastroprotective agent [12], antibacterial and anticancer [13]. However, studies are scanty about the *in vivo* actions of quercetin nano-formulations after oral administration. According to my knowledge, this research is the first to demonstrate the estrogenic effects of quercetin nano-formulations (how the daily oral administration reduces bone loss, dyslipidemia, oxidative stress, hyperglycemia and inflammation) in ovariectomized rats. Generally, this work is one of most recent researches to report the *in vivo* effect of quercetin-loaded phytosome nanoparticles (QP).

In light of the aforementioned information, the present survey was undertaken to establish the superiority of QP over the free form of quercetin by evaluating their actions as phytoestrogens. Consequently, the current study aims to evaluate physicochemical characterization and in-vitro antioxidant activity of QP, elucidate the biochemical abnormalities associated with experimental estrogen deficiency induced by ovariectomy surgery in albino rats and investigate the possible effects of free quercetin and QP in ovariectomized rats (OVX rats) in comparison with synthetic steroidal estrogen as a reference HRT.

2. Material and methods

2.1. Materials

Quercetin was obtained from Sigma-Aldrich chemie (GmbH, Germany). Phosphatidyl choline was a generous gift from Lipoid Company (Ludwigshafen, Germany). Estradiol benzoate (EB) was obtained from FOLONE® (5 mg/1 ml amp), Misr Pharmaceutical Company (Egypt). Cholesterol and all chemicals used in the current investigation are of analytical grade and provided from Sigma Chemical Co. (St. Louis MO, USA).

2.2. Methods

2.2.1. Preparation of quercetin-loaded phytosome nanoparticles (QP)

QP were prepared by thin film hydration method using molar ratio of quercetin, phosphatidyl choline and cholesterol (1:2:0.2), respectively [14]. The mixture was dissolved in 1:1 v/v of methanol: chloroform mixture in a round bottom flask and then evaporated in a rotary evaporator (Buchi Rotavapor® R-205, Germany) at 45 °C and 80 rpm under vacuum until obtaining thin film of dry lipid on the flask wall then left for time to ensure complete removal of the organic solvents. The film was then rehydrated by adding glucose 5% solution in rotary at 45 °C and 80 rpm under nitrogen gas stream until vesicles formation. To form small unilamellar vesicles of QP, sonication was carried out with probe sonicator (Ivymen, spain) for 5 min. Drug-free liposomes (FL) were prepared by the same procedure without quercetin.

2.2.2. Characterization of nanoparticles

2.2.2.1. Transmission electron microscopy (TEM). The morphology of the prepared nanoparticles was visualized by a negative stain electron microscopy method using high resolution TEM (Jeol JM-2100, Japan). One drop of sample was placed on a copper grid coated with carbon film and dried for 3–5 min on a filter paper. An aqueous solution of 2% tungstophosphoric acid was used as a negative staining agent. The grid was further dried by placing at room temperature, then loaded in the TEM and the areas were scanned for observation of vesicles [15].

2.2.2.2. Particle size analysis. Particle size distributions were measured using Zetasizer (Nano ZS, Malvern Instruments, UK). Zetasizer detects the back-scattered laser-light at 25 °C using laser diffraction technique. It reports the mean particle diameter and the polydispersity index (PDI) which is ranging from 0 (monodisperse) to 1 (polydisperse). The PDI value > 0.5 indicates a broad particle distribution [16]. All measurements were repeated five times at room temperature.

2.2.2.3. Zeta-Potential (ZP) measurements. ZP of prepared nano-formulations were determined using Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK). It was determined by measuring the direction and velocity that the particles moved in the applied electric field. The measurements of ZP were calculated as the average and standard deviation of measurements.

2.2.2.4. Spectroscopic characterization. The interaction of quercetin with liposome bilayer phospholipids was investigated by measuring the optical properties of quercetin, FL and QP using UV-VIS spectrophotometer (Jenway 6405, Barloworld Scientific, Essex, UK).

2.2.2.5. Determination of encapsulation efficiency. The encapsulation efficiency (EE %) of quercetin was determined by an indirect method [14]. Samples were centrifuged at 15,000 rpm for 60 min at 4°C in a refrigerated ultracentrifuge (SIGMA 3K30 Centrifuges, Germany). The free quercetin contents in supernatant were calculated from the calibration curve at 240 nm and the entrapped quercetin calculated as the difference between the total amount of the quercetin added to the preparation and the amount of unentrapped quercetin according to the following equation (Eq. (1)).

$$\mathbf{EE}\,\% = \frac{\mathbf{W(added\ drug)} - \mathbf{W(free\ drug)}}{\mathbf{W(added\ drug)}} * 100 \quad (1)$$

where W (added drug): the total amount of drug added to the preparation of phytosomes and W (free drug): The drug content in supernatant (unentrapped drug).

2.2.3. Determination of quercetin and quercetin-loaded phytosome antioxidant activity

Serial concentrations (5–100 μg/ml) of quercetin, QP and ascorbic acid were added to DPPH and the color was measured at 515 nm [17]. The antioxidant activity (AA %) was calculated as follows in Eq. (2).

$$\mathbf{AA}\,\% = 100 - \left\{ \frac{\left[\left(\mathbf{A_{Sample}} - \mathbf{A_{Blank\ sample}}\right) \times 100\right]}{\left(\mathbf{A_{Standard}} - \mathbf{A_{Blank\ standard}}\right)} \right\} \quad (2)$$

where: A_{Sample}: Absorbance of sample, $A_{Blank\ sample}$: Absorbance of blank sample, $A_{standard}$: Absorbance of standard and $A_{Blank\ standard}$: Absorbance of blank standard. Half maximal inhibitory concentration, IC_{50} (the amount in μg/ml required to reduce initial concentration of DPPH radicals by 50%) was calculated from the calibration curve.