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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.

2.2.4. Ovariectomy surgery

In the current survey, induction of menopausal symptoms was performed by bilateral ovariectomy under aseptic conditions [18]. Each rat was anesthetized by diethyl ether inhalation then hair was shaved and disinfected by 10% povidone-iodine. A midline dorsal skin incision, 3 cm long, approximately half way between the middle of the back and the base of the tail after placing the animal on its ventral surface. Incision of the muscles was made at linea alba. The ovary was found, surrounded by a variable amount of fat after accessing to peritoneal cavity. The blood vessels were ligated at the connection between the fallopian tube and uterine horn was cut and the ovary moved out. Muscle layer and skin suturing was performed by simple continuous suture using (3/0) antibacterial silk suture. Similarly, sham operation was performed using the same procedure except for the ligation and excision steps. Animals were given broad spectrum antibiotic (amoxicillin, 10 mg/kg, i. p) for 3 successive days after ovariectomy to eliminate the risk of postoperative infection.

2.2.5. In vivo experimental design

A total of 48 adult female albino rats aged 10–13 weeks old and weighing (120–150 g) were used. Rats were provided from the farm of National Organization for Drug Control and Research (NODCAR), Giza. They were housed in stainless steel cages at room temperature (24 ± 2 °C) with a 12 h light-dark cycle. They were fed on standard diet and tap water and were acclimatized to the environment for a week prior to experiment. All animal experiments were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). The animal studies were approved by the institutional guidelines for animal research and the protocols were previously certified by the Institutional Ethics Committee for Use of Animals from Faculty of Pharmacy (Girls), Al-Azhar University.

After one week acclimation period, 48 rats were randomly assigned to two main different groups;

I-Negative control, Sham group: 6 female rats were underwent sham operation.

II-Ovariectomized groups: 42 female rats were underwent bilateral ovariectomy operation. After two weeks from the surgery, rats were randomly assigned to 7 subgroups, 6 rats each as follows;

Group I (**OVX**)	Untreated ovariectomized rats served as positive control.
Group II (**EB**)	Ovariectomized rats were injected subcutaneously with (14 μg/100 g body weight) EB daily for 10 days [19].
Group III (**FL**)	Ovariectomized rats administered FL (p.o.), for 30 days.
Group IV (**Q_{10}**)	Ovariectomized rats received quercetin suspension (10 mg/kg, p.o.) in 0.5% gum acacia, daily for 30 days [20].
Group V (**Q_{50}**)	Ovariectomized rats administered quercetin suspension (50 mg/kg, p.o.) in 0.5% gum acacia, daily for 30 days [9].
Group VI (**QP_{10}**)	Ovariectomized rats received QP (10 mg/kg, p.o.) for 30 days.
Group VII (**QP_{50}**)	Ovariectomized rats administered QP (50 mg/kg, p.o.) for 30 days.

2.2.6. Biochemical analysis

2.2.6.1. Tumor necrosis factor alpha (TNF-α) measurement as an inflammatory marker. Using Rat TNF- α ELISA kit (IBL, Hamburg, Germany, Catalog No. (BE45471)) [21].

2.2.6.2. Determination of oxidative stress biomarkers. Erythrocytes reduced glutathione (GSH) [22] and malondialdehyde (MDA) [23] were determined colorimetrically.

2.2.6.3. Determination of bone markers. All bone markers {serum acid phosphatase (ACP) [24], alkaline phosphatase (ALP) [25], calcium [26] and phosphorus [27]} were determined colorimetrically by Bio diagnostic kits.

2.2.6.4. Determination of lipid profile. Serum triglycerides (TG) [28], total cholesterol (TC) [29], high-density lipoprotein (HDL-c) [30] and low-density lipoprotein (LDL-c) [31] were determined spectrophoto-metrically using enzymatic colorimetric Bio diagnostic kits. Very low-density lipoprotein (VLDL-c) concentration was calculated by means of the equation; VLDL-c = (Triglycerides/5) [32]. Atherogenic indices were also calculated (TC/HDL-c and LDL-c/HDL-c).

2.2.6.5. Determination of glucose concentration. Blood glucose concentration was determined using enzymatic colorimetric Bio diagnostic kits, Catalog No. (GL1320) [33].

2.2.6.6. Determination of weight gain. Weight gain was calculated as the difference between the final weight and the initial weight.

2.2.7. Statistical analysis of the data

Results were expressed as means ± S.E. Statistical evaluation was done using one-way analysis of variance and Tukey's multiple comparison tests using Statistical Package for the Social Sciences program (SPSS version 17.0, Chicago, USA). Values of $P < 0.05$ were considered significant.

3. Results

3.1. Physical characterization of the prepared nanoparticles

Surface morphological studies of prepared QP using TEM have indicated that the prepared nanoparticles were homogeneously spherical, well and uniformly dispersed nano-sized system (Fig. 1A). The hydrodynamic size of FL and QP measured by dynamic light scattering (DLS) were 55 ± 12.7 and 70 ± 7.44 nm, respectively as shown in Fig. 1B with PDI 0.6 and 0.2, respectively. The average ZP of FL and QP were −52.7 ± 3.8 mv and −44.6 ± 4.1 mv, respectively (Fig. 1C).

UV-VIS spectra of quercetin, QP and FL presented in Fig. 2 showed two absorption bands for free quercetin at 240 nm and the other band at 280 nm which disappeared after phytosome formation while the other band at 240 nm was slightly shifted to 235 nm. The encapsulation efficiency of QP calculated was very high (98.4%).

3.2. DPPH assay

Quercetin and QP exhibited a considerable antioxidant potential with IC_{50} values of 13.9 and 11.4 μg/mL, respectively when compared to ascorbic acid (5.18 μg/mL).

3.3. Effect on TNF-α

Table 1 elucidated that in OVX rats, the level of serum TNF-α was significantly ($p < 0.05$) increased by 1.5-folds as compared with sham operated rats. Although, it should be pointed out that levels of serum TNF-α were significantly decreased in rats receiving either Q (10 and 50 mg/kg), QP (10 and 50 mg/kg) or EB by 8.2, 15, 12, 18 and 25%, respectively versus untreated OVX rats.

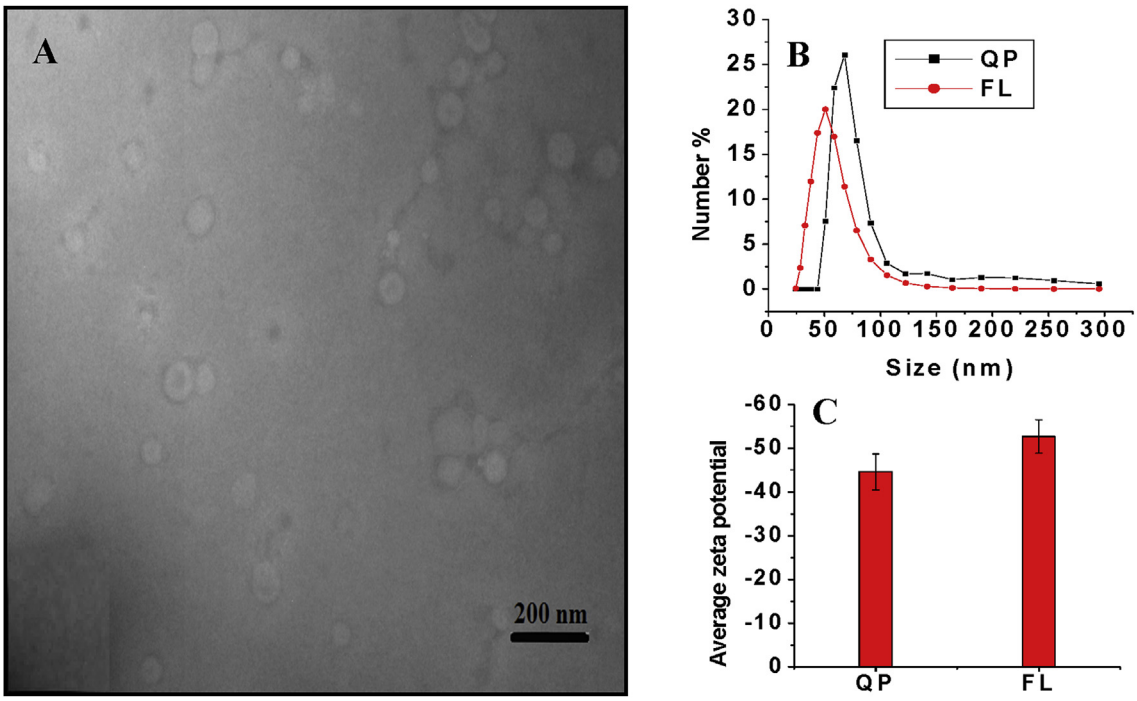

Fig. 1. (A) TEM image of quercetin-loaded phytosome showed homogeneous, spherical, well and uniform nanoparticles. (B) Particle size distribution of quercetin-loaded phytosome (QP) and free liposome (FL) measured by dynamic light scattering were 70 and 55 nm, respectively. (C) The average zeta potential of FL and QP were −52.7 ± 3.8 mv and −44.6 ± 4.1 mv, respectively.

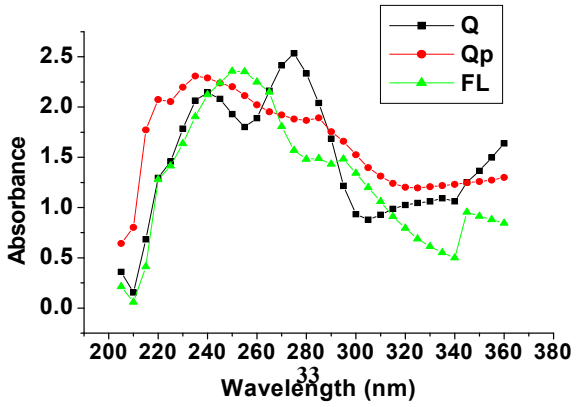

Fig. 2. UV-VIS spectra of quercetin (Q) (■), quercetin-loaded phytosome (QP) (●) and free liposome (FL) (▲) showed two absorption bands for free quercetin at 240 nm and the other band at 280 nm which disappeared after phytosome formation while the other band at 240 nm was slightly shifted to 235 nm.

3.4. Effect on oxidative stress biomarkers

Our results in Table 1 revealed that the blood reduced GSH content was significantly decreased by 2.2-folds while blood MDA level was significantly increased by 3.9-folds in OVX rats versus sham rats. On the contrary, the best oxidative stress prevention effect was obtained with EB, followed with high dose of QP (50 mg/kg) then low dose (10 mg/kg) of QP, followed by high dose of free quercetin (50 mg/kg).

3.5. Effect on bone markers

In our results as illustrated in Fig. 3(A and B), ACP and ALP activities in the OVX control group were significantly increased by 2.6 and 1.4-folds, respectively while serum calcium and phosphorus levels were significantly decreased (Fig. 3C and D) as compared to the sham group. No significant changes ($p > 0.05$) were observed in bone markers in rats treated with low dose of free quercetin (10 mg/kg) if compared to untreated OVX rats. In contrast, after four weeks of treatment with either Q (50 mg/kg), QP (10 and 50 mg/kg) or EB significantly ($P < 0.05$) lowered ALP and ACP activities while

Table 1
Effect of different treatments on TNF-α, MDA, GSH and body weight gain in comparison with estradiol benzoate in OVX-rats.

Rat groups	TNF-α (pg/ml)	MDA (nmol/ml)	GSH (mmol/L)	Body weight gain (g)
Sham	108.3 ± 2.17	12.44 ± 1.21	1364 ± 101	19.2 ± 2.94
Ovx	158.5 ± 3.01*	49.02 ± 1.05*	608.1 ± 40.2*	30.7 ± 3.37*
EB	119.7 ± 1.18#	17.26 ± 1.37#	1296 ± 53.0#	29.2 ± 2.50
FL	154.2 ± 1.99	49.19 ± 3.61	601.7 ± 46.7	31.2 ± 1.89
Q10	145.5 ± 1.18#	42.36 ± 0.31	735.7 ± 5.53	25.0 ± 1.65#
Q50	134.2 ± 0.72#	35.63 ± 0.58#	874.9 ± 21.2#	23.5 ± 2.53#
QP10	139.6 ± 1.30#	32.41 ± 0.85#	968.4 ± 20.7#	18.0 ± 1.24#
QP50	130.7 ± 1.18#	23.84 ± 0.82#	1258 ± 16.1#	11.0 ± 0.58#

Values are mean ± SE, n = 6 *: significantly different from sham-operated group and #: significantly different from OVX group at $p < 0.05$ level. EB: estradiol benzoate, FL: free liposome, Q: quercetin and QP: quercetin loaded phytosome. Differences among groups were analyzed by Tukey's multiple comparison tests after ANOVA.

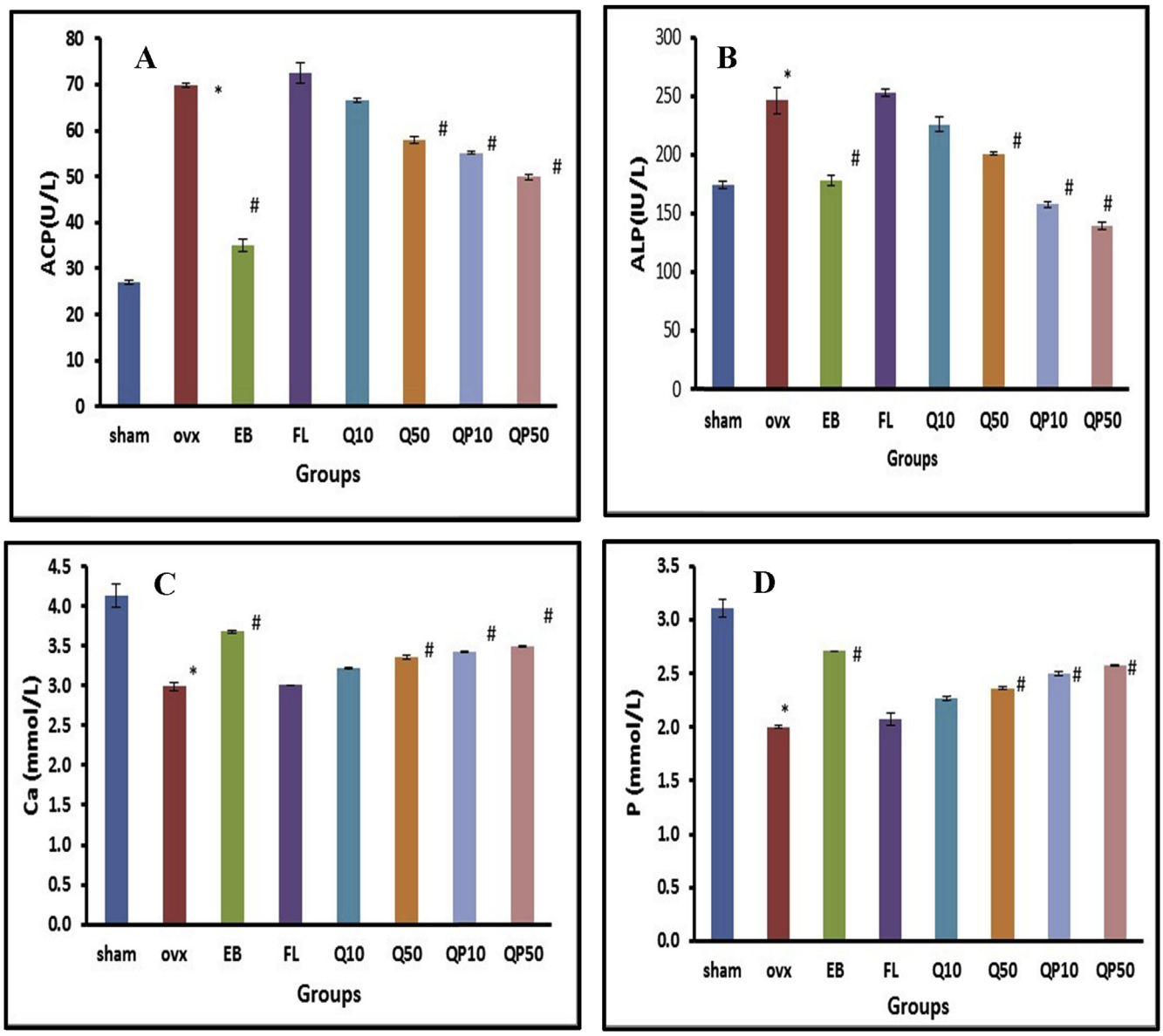

Fig. 3. Values of bone markers are mean ± SE, n = 6 *: significantly different from sham-operated group and #: significantly different from OVX group at $p < 0.05$ level. EB: estradiol benzoate, FL: free liposome, Q: quercetin and QP: quercetin loaded phytosome, (ACP): acid phosphatase, (ALP): alkaline phosphatase, (Ca): calcium and (P): phosphorus. Differences among groups were analyzed by Tukey's multiple comparison tests after ANOVA.

increased serum calcium and phosphorus levels were observed versus untreated OVX rats. Surprisingly, the best effect in reduction of ALP activity was obtained when the animals treated with higher dose (50 mg/kg) of QP that overtook EB effect.

3.6. Effect on lipid profile

OVX rats exhibited significant elevation in serum TG, TC, LDL-c, VLDL-c levels and atherogenic indices while significant reduction in HDL-c levels versus sham rats, as clarified in Fig. 4(A–E) and 5 (A-B). However, our data reported marked significant improvement of all lipid profile parameters and atherogenic indices after treatment with either Q (50 mg/kg**),** QP (10 and 50 mg/kg**)** or EB versus untreated OVX rats. Also, it should be pointed out that administration of low dose of Q (10 mg/kg) showed significant improvement of atherogenic indices and lipid profile expect for HDL-c levels as compared to untreated OVX rats.

3.7. Effect on blood glucose level

As clarified in Fig. 6, ovariectomy induced a significant increase in blood glucose level by 1.6-folds versus sham operated rats. However, treatment with either Q (10 and 50 mg/kg**)**, QP (10 and 50 mg/kg**)** or EB significantly ($p < 0.05$) reduced the blood glucose level by 19, 25, 32 and 37%, respectively as compared with untreated OVX rats.

3.8. Weight gain

Table 1 revealed that the weight gain was significantly increased ($p < 0.05$) by 1.6-folds in untreated OVX rats as compared with sham operated rats. On the other hand, the OVX rats administrated either Q (10 and 50 mg/kg) or QP (10 and 50 mg/kg) exhibited a significant decrease in weight gain ($P < 0.05$) by about 19, 23, 41 and 64%, respectively versus

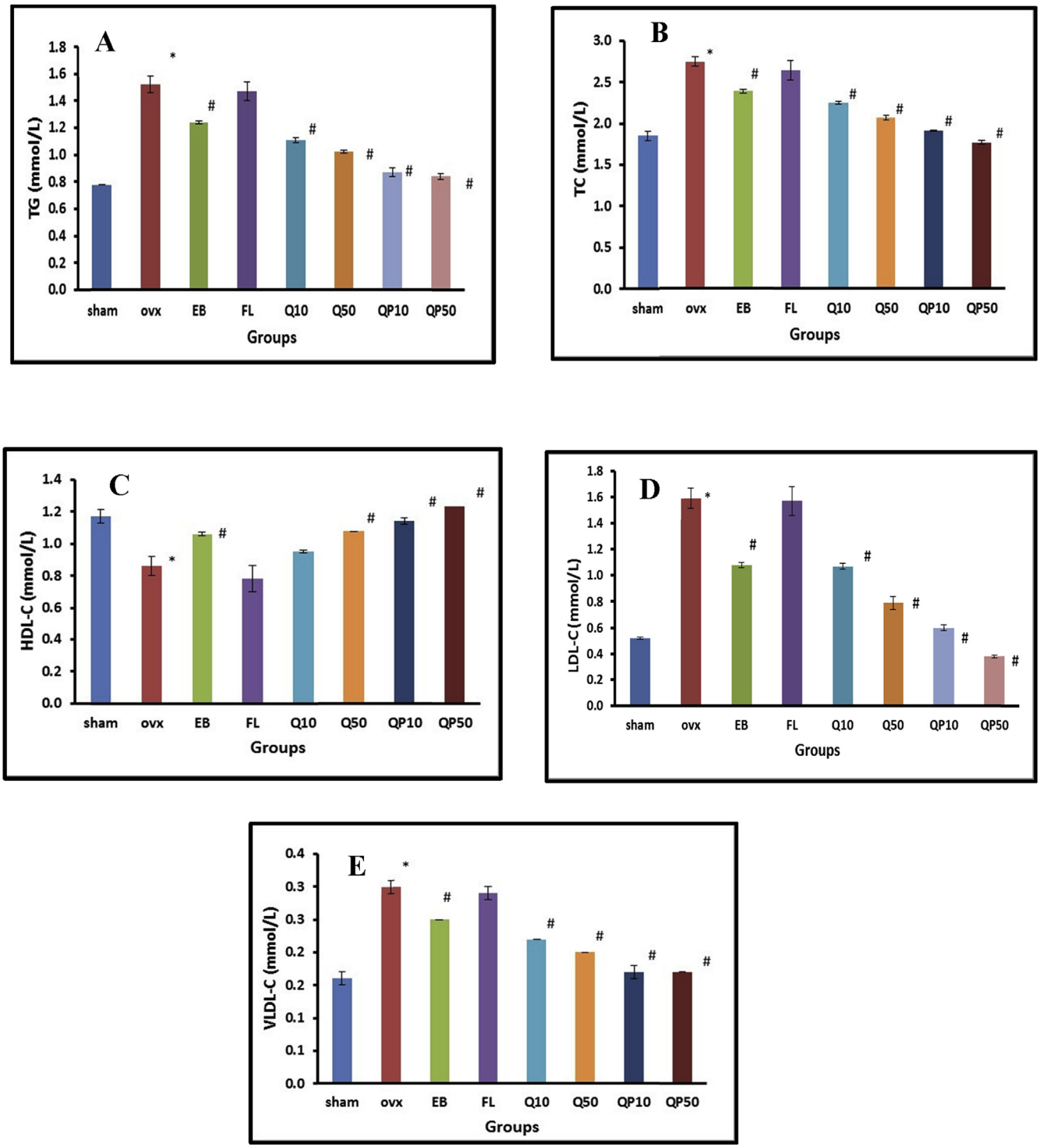

Fig. 4. Values of lipid profile are mean ± SE, n = 6 *: significantly different from sham-operated group and #: significantly different from OVX group at $p < 0.05$ level. EB: estradiol benzoate, FL: free liposome, Q: quercetin and QP: quercetin loaded phytosome, (TG): triglycerides, (TC): total cholesterol, (HDL-c): high-density lipoprotein, (LDL-c): low-density lipoprotein and (VLDL-c): very low-density lipoprotein. Differences among groups were analyzed by Tukey's multiple comparison tests after ANOVA.

untreated OVX rats, whereas EB showed no significant change.

4. Discussion

4.1. Physical characterization of the prepared nano-formulations

In this work, nanoparticles were successfully prepared using thin film hydration method with homogenous size, in the nanometer range and without aggregation. Our TEM result was parallel to previous study which presented nanometric spheres of quercetin loaded polymeric micelles [34]. DLS results revealed that the average diameter of phytosomes increased by 15 nm after loading of qurecetin, most likely due to addition of qurecetin and complex formation between qurecetin and phosphatidyl choline [35].

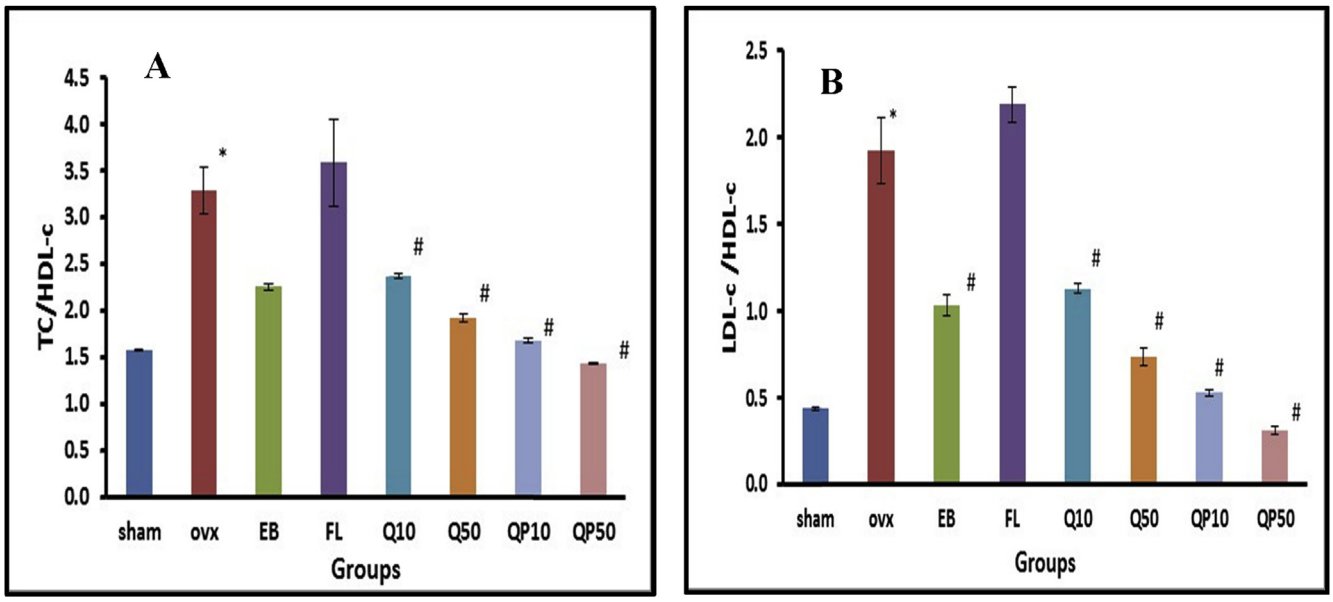

Fig. 5. Atherogenic indices values are mean ± SE, n = 6 *: significantly different from sham-operated group and #: significantly different from OVX group at $p < 0.05$ level. EB: estradiol benzoate, FL: free liposome, Q: quercetin and QP: quercetin loaded phytosome, (TC): total cholesterol, (HDL-c): high-density lipoprotein, (LDL-c): low-density lipoprotein and (TC/HDL-c and LDL-c/HDL-c): atherogenic risk ratios. Differences among groups were analyzed by Tukey's multiple comparison tests after ANOVA.

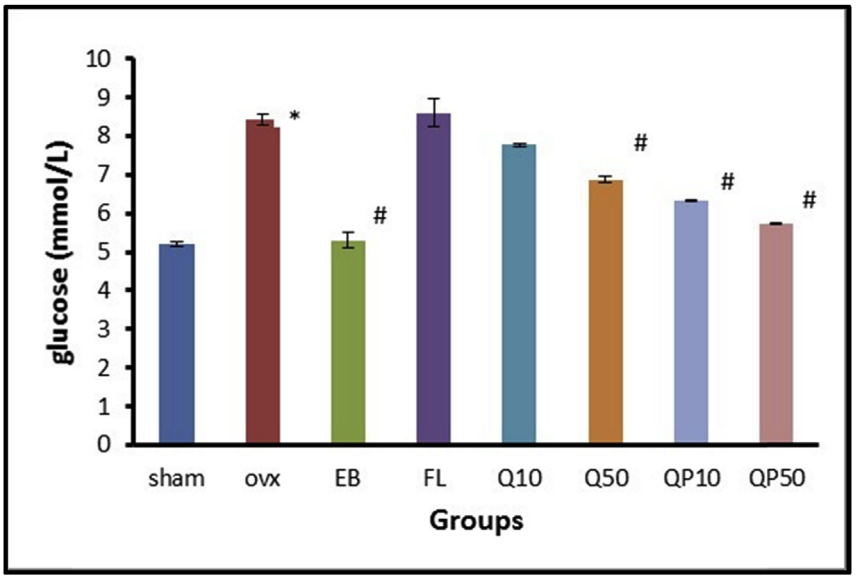

Fig. 6. Values of blood glucose are mean ± SE, n = 6 *: significantly different from sham-operated group and #: significantly different from OVX group at $p < 0.05$ level. EB: estradiol benzoate, FL: free liposome, Q: quercetin and QP: quercetin loaded phytosome. Differences among groups were analyzed by Tukey's multiple comparison tests after ANOVA.

Homogeneous and narrow size distribution of the prepared QP, confirmed by PDI results, assured that quercetin has high affinity to form phytosome with phosphatidyl choline due to its planar configuration [8].

The highly negative surface charge of the prepared QP contributes to high sample stability with the advantage of prolonged shelf storage [36]. The measured ZP of unloaded vesicles decreased when qurecetin was added to the plain liposome. This reduction of negativity may be attributed to hydrogen bond formation between OH- group of quercetin and polar part of phosphatidyl choline [14]. Moreover, from the QP UV-VIS spectrum, the disappearance of free quercetin band at 280 nm and the shifting of the other band at 240 nm indicated complex formation between phosphatidyl choline and quercetin.

The achieved high EE % indicated that the ratio of lipids and drug used is the optimal choice for QP preparation. These results were consistent with recent survey which found that the high EE % was attributed to quercetin planar configuration, which can be easily intercalated into the organized structure of phosphatidyl choline within the vesicle membranes, preventing quercetin from escaping [8]. Observed high DPPH scavenging ability of QP confirmed that the capacity of the phenolic group of quercetin to donate hydrogen for free radical scavenging was maintained after encapsulation. From the aforementioned characterization outcomes, it can be concluded that thin hydration method succeeded to form phytosomes that may be a promising nanodrug delivery system.

4.2. Effect on TNF-α

In the present study, it was observed that ovariectomy triggered an inflammatory response as evidenced by elevated levels of plasma TNF-α whereas all treatments for OVX rats significantly decreased it. The improvement effect was more pronounced in QP than free quercetin in dose-dependent manner. Previous studies supported our results and showed that quercetin decreased proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, whereas, it increased the anti-inflammatory cytokine IL-10 level in lipopolysaccharides-induced inflammation in mice [37]. The mechanism of quercetin anti-inflammatory effect was explained through the modulation of the NF-κB signal transduction cascade [38]. Furthermore, oral administration of quercetin-loaded microcapsules decreased neutrophil recruitment, reduced IL-1β and IL-33 production and prevented the reduction of IL-10 compared to the nonencapsulated drug [39].

4.3. Effect on oxidative stress biomarkers

The current work indicated that ovariectomy induced imbalance between reactive oxygen species (ROS) production and antioxidant defense system, resulting in oxidative stress and lipid peroxidation as shown by increased MDA content and marked decrease in GSH content. These changes were significantly improved by administration of either Q (50 mg/kg), QP (10 and 50 mg/kg) or EB versus untreated OVX rats. Previous study demonstrated that treatment with high dose (50 mg/kg) of quercetin markedly enhanced the activities of SOD and glutathione peroxidase while it significantly reduced the level of MDA [40]. Also, it was found that quercetin-loaded chitosan nanoparticles play a major role in eliminating free radicals in a dose dependent manner via increasing SOD, CAT, glutathione-*S*-transferase and GSH levels [41]. This high antioxidant activity of quercetin was attributed to the presence of three active functional groups in its structure: the *ortho*-dihydroxy (catechol) moiety in the B ring, the C2-C3 double bond in conjunction with 4-oxo function and the hydroxyl substitutions at positions 3, 5 and 7 [42].

4.4. Effect on bone markers

Untreated ovariectomized rats showed significant hypocalcaemia, hypophosphatemia and increased bone turnover rate (increased serum ALP and ACP), where bone resorption exceeded its formation, leading to bone loss. On the other hand, the bone protective effects of QP on OVX rats was evidenced by its potential increase in serum calcium, phosphorus levels while decrease in serum bone turnover markers in comparison with untreated OVX rats. Nevertheless, treatment with low dose of free quercetin (10 mg/kg) did not produce any significant effects due to weak effect of low dose poor absorbed quercetin for short duration but QP at the same dose significantly reduced risk of bone loss. The aforementioned data are in harmony with previous study which demonstrated that quercetin, as phytoestrogen, has structural similarities to estrogen conformation and binding capabilities to estrogen receptors which promote calcium absorption [43]. A possible mechanism by which quercetin protects against bone loss may be related to quercetin ability to: decrease osteoclastogenesis via inhibition of NF-κB ligand and activator protein-1, induce apoptosis of mature osteoclasts, inhibit the differentiation of osteoclast progenitor cells into preosteoclasts and enhance osteoblast proliferation, mineralization and activity [44].

4.5. Effect on lipid profile

In our model, QP at high dose (50 mg/kg) exerted obvious beneficial effects regarding ovariectomy-induced hyperlipidemia which overtook treatment with EB and free quercetin at the same dose as compared to untreated OVX rats. In agreement with our data, recent research emphasized that quercetin used alone or as iron complex clearly alleviated hyperlipidemia in obese rats and that quercetin–iron complexes showed better beneficial effects compared to quercetin taken alone [45]. They explained that quercetin acts by altering hepatic cholesterol absorption and triglyceride assembly and secretion as well as inhibition of phosphodiesterase in both adipose tissue and liver. Also, quercetin hypolipidemic effect could be referred to the ability to scavenge ROS which block TG secretion into the plasma and disturb cholesterol catabolism into bile acids. Indeed, quercetin alone and quercetin-iron complex decreased liver and adipose LPL and increased adipose HSL, suggesting fat storage reduction and lipolysis enhancement.

4.6. Effect on glucose concentration and body weight

The present work revealed significant increase in blood glucose and body weight in untreated OVX rats versus sham group. Recent appraisal professed that hyperglycemia is a characteristic of postmenopausal women, indicating that estrogen deficiency induces glucose intolerance, hyperlipidemia and greater body weight, and an increase in adipose tissue mass and adipocyte size [46]. However, it should be noticed that EB reversed hyperglycemia in OVX rat followed by high dose of QP (50 mg/kg) then its lower dose (10 mg/kg), and finally tailed by high dose of free quercetin (50 mg/kg). Our data is consistent with recent study which emphasized that quercetin used alone or as iron complex significantly alleviated the hyperglycemia [45]. The beneficial effect of quercetin on glycemia may work through different mechanisms such as; stimulation of glucose influx via GLUT4, augmentation of glucokinase activity, increasing the glucose liver uptake and inhibition of hepatic glycogenolysis and gluconeogenesis [42].

Our results clearly revealed higher beneficial effects of QP than free quercetin in reducing of weight gain induced by ovariectomy, in dose-dependent manner. These results are compatible with recent inquiry which proved that treatment with quercetin or quercetin-iron complexes induced a significant decrease in body weight and relative liver and adipose tissue weights in obese rats [45]. Quercetin effect in weight reduction may be due to better glycemic control with reduction of the compensatory lipolytic response and consequent normalization of triglyceridemia without modifying the hypercholesterolemia [42].

5. Conclusion

Nanoparticles were successfully prepared using thin film hydration method as confirmed by their characterization and physicochemical properties. Low dose of free quercetin (10 mg/kg) failed to produce significant results in most parameters, but QP at the same dose exhibited almost same ameliorating effects as free quercetin at high dose (50 mg/kg) in inflammation, oxidative stress, bone, lipid, blood glucose and weight gain parameters. High dose of QP (50 mg/kg) displayed better results than free quercetin at same dose and almost restored the normal state. This enhanced therapeutic efficacy of quercetin as antioxidant, anti-inflammatory, hypolipidemic and other beneficial effects obtained by phytosome formation may be due to better absorption of QP. Eventually, knowledge gained in this inquiry may open the door to pursuing phytosome nanotechnology as a viable strategy for quercetin delivery in many therapeutic applications.

Conflict of interest

The authors declare that they have no conflict of interest.

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