Pre-Clinical Evidence for the Anti-Obesity Potential of Quercetin and Curcumin Loaded Chitosan/PEG Blended PLGA Nanoparticles

Hanaa H. Ahmed, Soheir E. Kotob*, Ahmed A. Abd-Rabou, Hadeer A. Aglan, Gamal A. Elmegeed and Ola A. Mohawed

Hormones Department, Medical Research and Clinical Studies Institute, National Research Centre, Dokki, Giza, Egypt.

*Corresponding Author E-mail: soheir1010@hotmail.com

https://dx.doi.org/10.13005/bpj/2274

(Received: 28 September 2021; accepted: 18 November 2021)

This research aimed to formulate quercetin (Qu) and curcumin (CUR)-loaded PLGA NPs coated with chitosan (CS) & PEG and to explore their therapeutic effect against obesity in rats. Qu and CUR nanostructures were prepared and characterized by Zetasizer and TEM. Then, the formulated nanoparticles and their free counterparts were employed for mitigation of obesity in female rats. The size of NPs was in nanometer range with an average size distribution 307.9 nm for Qu NPs and 322.5 nm for CUR NPs. The Qu NPs and CUR NPs were appeared in the TEM image containing core in which the Qu or CUR was localized and surrounded by the coat of PLGA-CS-PEG. The Qu NPs exhibited negative zeta potential at -8.5 mV, while, CUR NPs exhibited positive zeta potential at +0.916 mV. Treatment with orlistat, free Qu, Qu NPs, free CUR or CUR NPs elicited significant decline in body weight, BMI and Lee index. Orlistat and CUR NPs significantly diminished liver, heart and visceral adipose tissue weights. Furthermore, the suggested treatments significantly reduced the gonadal and subcutaneous adipose tissue weights. Orlistat significantly lessened kidney and adrenal weights. All treatments significantly minimized serum Chol., TG, LDL, glucose, INS, HOMA-IR, LH, MDA, TLR4 and NF-κB levels and elevated serum HDL, E2 and TAC levels. Orlistat significantly enhanced serum IL-10 level. Conclusively, Qu and CUR nanoformulations offer anti-obesity potency through their hypolipidemic, hypoglycemic, antioxidative and anti-inflammatory effects. Both Qu and CUR NPs manifested superior effect than their free counterparts, may be because of solubility elevation as well as bioavailability of the nanoencapsulation.

Keywords: Anthropometric Measurements; Biochemical Markers; Curcumin Nanoparticles; Lipid Profile; Obesity; Quercetin Nanoparticles.
resistance. Obesity is usually linked with several life-threatening ailments like hypertension, type 2 diabetes, cancer, respiratory complications, coronary heart disease, inflammation, and osteoarthritis.

Obesity, is identified as a global epidemic since it affects virtually all age and social classes in both developed and developing nations. It influences 1.5 billion humans globally, and is mostly a sequel of deleterious lifestyle preferences such as consumption of overeating food and paucity of exercise.

The available medications for obesity depends on lifestyle adjustments, decreasing energy consumption and augmenting energy expenditure. Other than lifestyle changes like exercise and diet adjustment, the present clinical approaches to handle obesity give primacy to pharmacotherapy, comprising the drugs, lorcaserin and orlistat, and surgical interventions such as bariatric surgeries. Whilst, these classical treatments have many side effects which desire their enhancement.

Utilization of a assortment of phytochemicals has protruded as an auspicious prospect for battling obesity and its comorbidities. A plenty of in vitro as well as in vivo researches have indicated that phytochemicals have anti-obesity response via restraining adipocyte proliferation and preadipocyte differentiation as well as potentiating apoptosis of adipocyte, dampening lipogenesis, lipolysis promotion as well as oxidation of fatty acid beta, and strengthening the regulatory networks controlling inflammation.

3,32,42,5,7-pentahydroxyflavone (Quercetin; Qu) is a secondary metabolite existed plentifully in a wide diversity of foods, like vegetables, fruits and beverages. Accumulating evidence demonstrated that Qu can conserve against obesity and its accompanied metabolic disorders. The possible beneficial role of Qu was ascertained by its numerous bioactivities, comprising antioxidant, anti-inflammatory and anti-obesity impacts as well as other prospective biological characteristics. Etxeberria et al. recorded that Qu diminishes the serum INS level and prevents the gain of body weight in high-fat sucrose administered rats.

The prime curcuminoid polyphenolic compound of the spice turmeric (Curcuma longa) is called curcumin (CUR); comprising 2–8% of the turmeric. Turmeric has well known biological activities like anti-inflammatory, anti-obesity, anti-oxidant anti-angiogenesis, and anti-carcinogenesis. The anti-obesity effect of CUR stems from hindering the differentiation of preadipocyte, defeating lipogenesis, enhancing oxidation of fatty acid, and minimizing adipose tissue inflammatory response.

Despite multiple health benefits, the commonly weak aqueous solubility, bioavailability, stability, and target specificity of phytochemicals, have restricted their clinical applications. Currently, nano-drug delivery tactic offers considerable promise for boosting the pharmacological properties of phytochemicals through augmenting their solubility and stability, elevating their bioavailability, protecting them from premature degradation in the body, prolonging their circulation time, and consequently intensifying their anti-obesity potential.

The current approach was constructed to examine the advanced impact of Qu and CUR loaded chitosan/polyethylene glycol (PEG) blended poly D,L-lactide-co-glycolide (PLGA) nanoparticles on obesity induced via HFD and explored the mechanism of action of these nanoparticles and related signalling pathways, seeking to provide experimental basis for the development of clinical application of this type of treatment in patients.

MATERIALS AND METHODS

Materials

Quercetin >95%, Curcumin >95%, poly D,L-lactide-co-glycolide (PLGA, ratio of copolymer 50:50, Inherent viscosity/ =/ 0.41), polyvinyl alcohol (PVA), chitosan (CS; MW 60–90/ kDa, deacetylation degree / =/ 85%), polyethylene glycol (2/ kDa, PEG), dichloromethane, glacial acetic acid and Tween 80 were acquired from Sigma–Aldrich (St. Louis, MO). The remain chemicals were of analytical grade purchased from Sigma–Aldrich (St. Louis, MO).

Preparation of Qu and CUR PLGA-NPs surface modified with chitosan and PEG

Quercetin and CUR-loaded PLGA NPs were synthesized according to the procedure described by Abd-Rabou and Ahmed, and Arya et al. with tiny alterations. In 3/ ml of
dichloromethane, 100/ mg of PLGA polymer & 10/ mg of quercetin or curcumin were dissolved. In 1% glacial acetic acid, 5/ ml of chitosan solution (12% w/v) was made, filtered & then titrated to 5/ ml of PVA aqueous solution (2% w/v) before emulsification. Thereafter, PEG (2/ kDa), 2% w/v, was provided to the prepared chitosan-PVA aqueous solution prior to emulsification to gain PEG-coated (PLGA-CS-PEG) NPs. In their organic phase PLGA and Qu or CUR were emulsified in the previous mentioned chitosan-PEG-PVA aqueous solution, then sonicated by a microtip probe sonicator (VC 505, Vibracell Sonics, Newton, USA) adjusted at energy output of 55/ W in an ice bath for 2/ min. The organic solvent was evaporated by stirring the emulsion at room temperature on a magnetic stirring plate overnight. In the next day, ultracentrifugation at 35,000/ rpm at 4/ °C for 20/ min (Sorvall Ultra-speed Centrifuge, Kendro) was done to obtain the formed NPs, then the unbound PVA, chitosan, PEG and un-encapsulated Qu or CUR were removed by double distilled water washing.

**Methods**

**Physicochemical characterization of Qu and CUR NPs**

**Measurement of particle size analysis & zeta potential**

Particle size and size distribution (polydispersity index) of Qu and CUR NPs were analyzed using dynamic light scattering (DLS) using a Zetasizer (Nano ZS, Malvern Instrument, UK). Zeta potential of the Qu and CUR NPs was also investigated using the same Zetasizer. <“ 1/ mg/ml of NPs suspension in MilliQ water was sonicated for 30/ sec in an ice bath using a sonicator (VC 505, Vibracell Sonics, Newton) set at 55/ W of energy output. 100 ìl of these NPs suspensions was diluted to 1/ ml in MilliQ water and used for particle size and zeta potential measurements.

**Transmission electron microscopic (TEM)**

Particle morphology of the synthesized nanoparticles was tested using transmission electron microscopy (TEM; JEOL JEM-2100, Japan). Briefly, into a Formvar-coated copper grids 100 ìg/ml of the nano-suspensions were dropped, then after drying complete, the samples were stained by uranyl acetate, 2% w/v, (Electron Microscopy Services, Ft. Washington, PA). Digital Micrograph and Soft Imaging Viewer Software was applied for image capture & analysis wer.

**In vivo study**

**Animal handling**

A 64 adult female *Wistar strain* albino rats of, with body weight of 130 ± 10 g and age of 90 d, were applied in the current experimental setting. The rats were comes from the Animal Facility Unit of the National Research Centre, Dokki, Giza, Egypt and placed in cages of polypropylene (8 rats/cage) in ideal environmental conditions, clean and controlled air room, a temperature of 24±1°C, a 12/12 hrs light/dark cycle, a 60±5% of relative humidity & free entrance to tap water as well as standard rodent chow. Rats were left to adapt to these conditions for 2 weeks before the experiment beginning.

**Ethical Statement**

Housing and management of animals followed the care and use of laboratory animals recommendations and guidelines. The research was done in compliance with the code of ethics of the World Medical Association (Helsinki Declaration) for animal experiments as well as the experimental protocol was agreed by the institution Ethical Committee for Medical Research, National Research Centre, Egypt (Code No:18175).

**Experimental groups**

Next to the period of acclimatization, according to Buettner et al.22 a standard rodent chow, with protein of 26.5%, fat of 3.8%, carbohydrate of 40%, and crude fiber of 4.5% in 100 g of chow, was administered to rats (eight rats) for the experimental period (18 weeks) and deemed as the lean control group (control group). Obese model was generated in the other 56 rats by the 12th weeks of HFD feeding (protein of 19.93%, cholesterol of 15%, carbohydrate of 57.50%, and dietary fiber of 2.81% in 100 g of chow according to Soliman et al.23 method (Obese group). The obese group was categorized into 7 groups (8 rats/group). (1): untreated obese group; (2): obese rats treated with orlistat orally (200 mg/kg b.wt.) every day in accordance with Nishioka et al.24 for 6 weeks; (3): obese rats orally administered 1 ml/rat of PLGA-CS-PEG nano-void daily for 6 weeks; (4) and (5): obese rats supplemented orally with freeQu or Qu NPs respectively in a dose of 10 mg/kg b.wt. dissolved in 1 ml distilled water/rat25 every day for
6 weeks; (6) & (7): obese rats orally received free CUR or CUR NPs respectively (20 mg/kg b.wt.) dissolved in 1 ml distilled water/rat daily for 6 weeks.

**Anthropometric measurements**

After final administration, the existence of obesity was assessed using Lee index according to the equation of Bernardis and Patterson:

\[
\text{Lee index} = \sqrt[3]{\text{body weight (g) / nose-to-anus length (cm)}}.
\]

The immediately anterior to the forefoot (abdominal circumference (AC)), immediately behind the foreleg (thoracic circumference (TC)), and nose-to-anus or nose-anus length (body length) were also determined. Also, body mass index (BMI) was measured.

\[
\text{BMI (g/cm}^2) = \frac{\text{Body weight (g)}}{\text{Length}^2 (\text{cm}^2)}
\]

according to Novelli et al was determined.

**Blood and tissue sampling**

After taking the anthropometric parameters, the rats were fasted for 12-14 hrs (overnight), & the samples of blood were collected, under anesthesia of diethyl ether, from the tail vein. The blood specimens were collected in a centrifuge tube in the absence of any agent of anticoagulant, then the samples left for 45 minutes to coagulate at room temperature to collect the serum. The samples were separated using centrifugation for 15 minutes at 1800 × g and 4°C by the cooling centrifuge apparatus. Serum samples were frozen and stored at −20°C for further biochemical analyses. After blood samples collection, the rats were euthanized using the cervical dislocation technique.

Liver, kidneys (right and left), heart, spleen, adrenal, thymus, and visceral adipose tissue (VAT), gonadal adipose tissue (GAT) as well as subcutaneous adipose tissue (GAT) were removed according to the defined anatomical landmarks. The dissected organs and tissues were washed with isotonic saline, blotted dry and weighed immediately.

**Biochemical determinations**

Serum total cholesterol (Chol), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were assessed using colorimetric method through the kits acquired from Reactivos GPL Co. (Barcelona, Spain) according to Meiatinni methods, Bucolo and David and Naito respectively. Serum glucose and malondialdehyde (MDA) concentrations were assigned colorimetrically applying kits purchased from Biodiagnostic (Egypt) following Trinder and Ohkawa et al methods respectively.

Quantitative analysis of serum insulin (INS) level was performed using ELISA kit secured from Immunoscopec Co. CA (Netherlands) following the National Committee for Clinical Laboratory Standards Procedures for the collection of diagnostic blood specimens by venipuncture method. Estimation of insulin resistance was done through applying the method of homeostasis model assessment, HOMA-IR, and was determined using the following formula:

\[
\text{Serum glucose (mg/dl) × fasting serumINS (MIU/mL) divided by 405}
\]

Serum leutinizing hormone (LH) level was evaluated using ELISA kit obtained from Immunoscopec Co. CA (Netherlands) following Uotilla et al. method. Moreover, assessment of the serum estradiol (E2) level was carried out using ELISA kit provided from Biocheck, Inc, (USA) according to Tietz method. Serum total antioxidant capacity (TAC), nuclear factor kappa B (NF-kB) as well as interleukin 10 (IL-10) levels were quantified by ELISA using kits supplied from Sino Gene Clon Biotech. Co., Ltd. (China) following the manufacturer’s manual. Finally, serum toll–like receptor 4 (TLR4) level was assessed using ELISA kit procured from Sunlong Biotech. Co., Ltd. (China) following the operating instructions.

**Statistical analysis**

The results of the experiment were illustrated as arithmetic means and their standard errors (SE) (Mean±SE). The analysis of data were done by one-way analysis of variance using the Statistical Package for the Social Sciences program (SPSS), version 17 followed by Bonferroni post hoc test to compare significance among the studied groups. In all analyses, p<0.05 was taken to improve statistical significance.

**RESULTS**

**Physicochemical characterization of Qu and CUR NPs**

The particle size distribution as well as zeta potential of the formulated NPs were
specified using dynamic light scattering (DLS) by a Zetasizer, whereas the morphology of NPs was detected by TEM. In this setting, our findings indicated that the designed NPs were of nanometer size range with an average size distribution 307.9 nm for Qu NPs (Fig. 1A) and 322.5 nm for CUR NPs (Fig. 2A).

The Qu NPs configuration was appeared in the TEM image containing core in which the Qu was localized and surrounded by the coat of PLGA-CS-PEG (Fig. 1B). The zeta potential of Qu NPs was illustrated in Fig. (1C), which demonstrated the negative zeta potential at -8.5 mV. On the other hand, the CUR NPs pattern was seen in the TEM image containing core in which the CUR was centralized and enclosed by the coat of PLGA-CS-PEG (Fig. 2B). The zeta potential of CUR NPs was represented in Fig. (2C), which revealed the positive zeta potential at +0.916 mV.

**Effect of orlistat, free Qu, Qu NPs, free CUR or CUR NPs on anthropometrical parameters**

The results in Table (1) evaluated the influence of treatment with orlistat, free Qu, Qu NPs, free CUR or CUR NPs on anthropometric parameters of obese rats. Prolonged feeding of rats with HFD provoked significant (p<0.05) elevation in the body weight, TC, AC, BMI and Lee index versus the control group. Obese rats treated with orlistat elicited significant (p<0.05) reduction in body weight, TC, AC, BMI and Lee index in comparison with the untreated obese rats. Meanwhile, treatment of obese rats with free

![Fig. 1. Qu NPs characterization. A] Size distribution of Qu (307.9 nm) using DLS by a Zetasizer. B] Morphology of Qu using TEM by Digital Micrograph and Soft Imaging Viewer Software. C] Zeta potential (negative charge on the Qu NPs surface -8.5 mV)
Qu, Qu NPs, free CUR and CUR NPs evoked significant ($p<0.05$) detraction in body weight, BMI and Lee index relative to the untreated obese rats. Additionally, all treated groups experienced insignificant ($p > 0.05$) change in body length and TC comparing with the untreated obese rats. Obese rats treated with free Qu, Qu NPs and free CUR exhibited insignificant ($p > 0.05$) change in AC while those treated with CUR NPs recorded significant ($p<0.05$) lowering in AC versus the untreated obese rats.

The obese groups treated with PLGA-CS-PEG nano-void or free Qu displayed significant ($p<0.05$) enhancement in weight gain versus the obese group treated with orlistat. Moreover, obese rats treated with PLGA-CS-PEG nano-void exhibited significant ($p<0.05$) increase in BMI and Lee index when compared with the obese group treated with orlistat.

**Effect of orlistat, free Qu, Qu NPs, free CUR or CUR NPs on different organs and tissues weight**

The obtained data in Table (2) illustrated the effect of treatment with orlistat, free Qu, Qu NPs, free CUR or CUR NPs on the weight of different organs and tissues of obese rats. Prolonged feeding of rats with HFD brought about significant ($p<0.05$) enhancement in the weight of the liver, left kidney, heart, adrenal, thymus and visceral, gonadal as well as subcutaneous adipose tissues versus the control rats. However, supplementation of HFD-fed rats with orlistat or CUR NPs inspired significant ($p<0.05$) drop in liver, heart and visceral, gonadal as well as subcutaneous adipose tissues weight in comparison with those left without treatment. Also, treatment of obese rats with orlistat(5,3),(994,994)

![Fig. 2. CUR NPs characterization. A] Size distribution of CUR (322.5 nm) using DLS by a Zetasizer. B] Morphology of CUR using TEM by Digital Micrograph and Soft Imaging Viewer Software. C] Zeta potential (positive charge on the CUR NPs surface +0.916 mV)](image-url)
treated with Qu NPs exhibited significant (p<0.05) depletion in visceral, gonadal and subcutaneous adipose tissues weight relative to the untreated obese rats. Treatment of obese rats with free Qu or free CUR caused significant (p<0.05) drop in the weight of gonadal and subcutaneous adipose tissues contrary to those left without treatment. The group of obese rats treated with PLGA-CS-PEG nano-void revealed significant (p<0.05) elevation in the weight of the visceral, gonadal and subcutaneous adipose tissues in comparison with that treated with orlistat. Moreover, the obese rats in the groups treated with free Qu or free CUR manifested significant (p<0.05) rise in the weight of gonadal adipose tissues versus the obese rats in the group treated with orlistat.

**Effect of orlistat, free Qu, Qu NPs, free CUR or CUR NPs on serum lipid profile**

The represented data in Table (3) showed the influence of treatment with orlistat, free Qu, Qu NPs, free CUR or CUR NPs on serum lipid profile of obese rats. Feeding rats with HFD for a long period of time induced significant (p<0.05) promotion in Chol, TG and LDL serum levels in concomitant with significant (p<0.05) drop in HDL serum level by contrast with the control rats. While, treatment of obese rats with orlistat, free Qu, Qu NPs, free CUR or CUR NPs produced significant (p<0.05) decline in Chol, TG and LDL serum levels along with significant (p<0.05) rise in HDL serum level versus those left without treatment. The group of obese rats treated with PLGA-CS-PEG nano-void exhibited significant (p<0.05) enhancement in Chol, TG and LDL serum levels along with significant (p<0.05) diminution in HDL serum level in contrary to the group of obese rats treated with orlistat.

**Effect of orlistat, free Qu, Qu NPs, free CUR and CUR NPs on serum glucose, INS and HOMA-IR values**

Table (4) demonstrated the influence of treatment with orlistat, free Qu, Qu NPs, free CUR or CUR NPs on serum glucose, INS and insulin resistance values of obese rats. Extended feeding of rats with HFD provoked significant (p<0.05) advancement in glucose, INS serum levels and HOMA-IR value relative to the control rats. On the opposite side, treatment of obese rats with orlistat, free Qu, Qu NPs, free CUR or CUR NPs brought about significant (p<0.05) drop in glucose, INS serum levels and HOMA-IR values versus the untreated obese rats. In comparison with the group of obese rats receiving orlistat, the HFD-fed rats treated with PLGA-CS-PEG nano-void, free Qu or free CUR displayed significant (p<0.05) rise in glucose serum level. Moreover, the groups of obese rats treated with PLGA-CS-PEG nano-void or free Qu exhibited significant (p<0.05) elevation in

| Parameters Groups | Body weight(g) | Body length(cm) | TC (cm) | AC (cm) | BMI (g/cm^2) | Lee Index |
|-------------------|----------------|-----------------|--------|---------|-------------|-----------|
| Control group     | 129.3±3.05     | 20.31±0.31      | 11.12±0.23 | 12.50±0.23 | 0.31±0.01   | 0.249±0.004 |
| Obese group       | 275.2±4.09     | 21.17±0.24      | 12.24±0.16 | 14.43±0.24 | 0.61±0.02   | 0.312±0.002 |
| Ob+Orlistat       | 135.0±4.22     | 20.52±0.29      | 11.56±0.14 | 13.20±0.28 | 0.33±0.01   | 0.256±0.009 |
| Ob+PLGA-CS-PEG nano-void | 263.1±7.61 | 20.92±0.35     | 12.02±0.10 | 14.06±0.35 | 0.58±0.02   | 0.298±0.009 |
| Ob+free Qu        | 161.4±3.62     | 20.81±0.13      | 11.80±0.07 | 13.65±0.14 | 0.37±0.01   | 0.273±0.004 |
| Ob+Qu NPs         | 147.5±5.90     | 20.67±0.17      | 11.75±0.09 | 13.50±0.16 | 0.346±0.02  | 0.267±0.009 |
| Ob+free CUR       | 153.7±5.96     | 20.70±0.21      | 11.76±0.08 | 13.61±0.16 | 0.358±0.01  | 0.270±0.004 |
| Ob+CUR NPs        | 141.0±3.40     | 20.57±0.22      | 11.66±0.11 | 13.34±0.15 | 0.33±0.01   | 0.260±0.005 |

Data were depicted as Mean ± S.E for each group of rats n=8.
a: Change of significant at P<0.05 comparing with the control rats.
b: Change of significant at P<0.05 comparing with the obese rats.
c: Change of significant at P<0.05 comparing with the rats treated with orlistat.
Table 2. Effect of treatment with orlistat, free Qu, Qu NPs, free CUR or CUR NPs on weight of different rat’s organs of obese rats

| Parameters Groups | Liver (g) | Kidney (g) | Heart (g) | Spleen (g) | Adrenal (g) | Thymus (g) | Adipose tissue (g) |
|-------------------|----------|------------|-----------|------------|------------|------------|-------------------|
|                   | R        | L          |           |            |            |            | Visceral          |
| Control group     | 7.16±0.07| 0.81±0.03  | 0.78±0.02 | 0.76±0.03  | 0.15±0.01  | 0.20±0.01  | 1.8±0.05          |
| Obese group       | 8.71±0.33 | 0.93±0.05  | 0.92±0.05 | 0.89±0.03  | 0.34±0.03  | 0.35±0.04  | 3.4±0.22          |
| Ob+ orlistat      | 7.61±0.14 | 0.83±0.02  | 0.80±0.02 | 0.78±0.02  | 0.25±0.01  | 0.25±0.01  | 2.3±0.04          |
| Ob+ PLGA-CS-PEG nano-void | 8.31±0.16 | 0.90±0.03  | 0.90±0.02 | 0.85±0.03  | 0.32±0.02  | 0.31±0.03  | 3.2±0.18          |
| Ob+ free Qu       | 7.91±0.17 | 0.87±0.02  | 0.87±0.02 | 0.84±0.02  | 0.30±0.01  | 0.30±0.01  | 2.8±0.15          |
| Ob+ Qu NPs       | 7.77±0.18 | 0.86±0.03  | 0.84±0.03 | 0.81±0.05  | 0.28±0.02  | 0.27±0.02  | 2.6±0.15          |
| Ob+ free CUR      | 7.80±0.22 | 0.86±0.03  | 0.86±0.02 | 0.83±0.03  | 0.29±0.02  | 0.29±0.01  | 2.7±0.16          |
| Ob+ CUR NPs      | 7.66±0.23 | 0.84±0.02  | 0.81±0.01 | 0.82±0.03  | 0.27±0.01  | 0.26±0.02  | 2.4±0.24          |

Data were depicted as Mean ± S.E for each group of rats n=8.
a: Change of significant at P<0.05 comparing with the control rats.
b: Change of significant at P<0.05 comparing with the obese rats.
c: Change of significant at P<0.05 comparing with the rats treated with orlistat.
and IL-10 levels of obese rats. Feeding rats with HFD for a long period of time elicited significant (p<0.05) rise in TLR4 and NF-kB serum levels in association with significant (p<0.05) reduction in IL-10 serum level when compared to the control rats. However, treatment of obese rats with orlistat, free Qu, Qu NPs, free CUR or CUR NPs instigated significant (p<0.05) decline in serum TLR4 and NF-kB levels relative to the untreated obese rats. Simultaneously, the group of obese rats treated with orlistat manifested significant (p<0.05) enhancement in IL-10 serum level in contrary to the group of untreated obese rats. Furthermore, treatment of obese rats with PLGA-CS-PEG nano-void caused significant (p<0.05) elevation in TLR4 and NF-kB serum levels versus the group of obese rats treated with orlistat.

**DISCUSSION**

Phytochemicals encapsulation into polymeric nanoparticles or conjugation to polymers

| Table 3. Effect of treatment with orlistat, free Qu, Qu NPs as well as free CUR and CUR NPs on lipid profile of obese rats |
|---------------------------------------------------------------|
| **Parameters** | **Chol (mg/dL)** | **TG (mg/dL)** | **HDL (mg/dL)** | **LDL (mg/dL)** |
| Groups | | | | |
| Control group | 69.2±1.2 | 83.1±2.8 | 35.5±2.7 | 23.2±1.4 |
| Obese group | 156.2±5.9a | 128.3±5.9a | 22.9±0.88a | 93.8±5.7a |
| Ob+Orlistat | 75.9±1.9b | 87.2±2.2b | 33.9±0.9b | 32.7±3.0b |
| Ob+ PLGA-CS-PEG nano-void | 150.1±3.6c | 121.6±1.4c | 25.1±0.9c | 89.5±1.9c |
| Ob+free Qu | 88.0±3.3b | 97.0±2.2b | 30.1±1.5b | 41.5±2.4b |
| Ob+ Qu NPs | 79.2±2.6b | 94.30±6.4b | 31.6±1.4b | 38.5±1.1b |
| Ob+free CUR | 83.3±2.5b | 96.2±3.5b | 30.8±1.1b | 39.0±0.9b |
| Ob+CUR NPs | 77.3±1.9b | 90.7±2.4b | 32.9±1.2b | 34.9±2.1b |

Data were depicted as Mean ± S.E for each group of rats n=8.
a: Change of significant at P<0.05 comparing with the control rats.
b: Change of significant at P<0.05 comparing with the obese rats.
c: Change of significant at P<0.05 comparing with the rats treated with orlistat.

| Table 4. Effect of treatment with orlistat, free Qu, Qu NPs as well as free CUR and CUR NPs on serum glucose, INS and insulin resistance values of obese rats |
|---------------------------------------------------------------|
| **Parameters** | **Glucose (mg/dL)** | **INS (MIU/ml)** | **HOMA-IR** |
| Groups | | | |
| Control group | 69.8±1.7 | 13.5±0.14 | 2.3±0.07 |
| Obese group | 153.7±1.4a | 27.8±0.12a | 10.5±0.13a |
| Ob+Orlistat | 73.0±2.0b | 14.6±0.83b | 2.6±0.19b |
| Ob+ PLGA-CS-PEG nano-void | 150.1±4.3c | 25.4±1.12c | 10.2±0.35c |
| Ob+free Qu | 88.3±1.7bc | 19.3±0.91bc | 4.2±0.17bc |
| Ob+ Qu NPs | 83.8±2.1b | 17.5±1.33b | 3.6±0.28b |
| Ob+free CUR | 86.9±2.1bc | 18.6±1.25bc | 3.9±0.24bc |
| Ob+CUR NPs | 79.5±3.6b | 15.0±0.54b | 2.9±0.13b |

Data were depicted as Mean ± S.E for each group of rats n=8.
a: Change of significant at P<0.05 comparing with the control rats.
b: Change of significant at P<0.05 comparing with the obese rats.
c: Change of significant at P<0.05 comparing with the rats treated with orlistat.
enhance their bioavailability and augment their sustained and controlled release rate in addition to the enhancement of their aqueous solubility, and stability. The polymeric nanoparticles with their distinctive surface chemistry can adsorb, entrap, or covalently attach medicinal phytochemicals to provide a considerable delivery enhancement. The synthetic polymers [poly(lactide-coglycolide) (PLGA), poly (lactide) (PLA), poly (δ-caprolactone) (PCL) and poly (ethylene glycol) (PEG)] are the most common used biodegradable polymers. The most common natural polymers include chitosan and alginate. PLGA nanoparticles have acquired prominent attention as an effective delivery vehicle, owing to its ease of preparation, elevated the ability of drug loading, competence of exceptional endocytosis, and controlled drug release manner. Another frequently applied biocompatible polymer is PEG, which is extremely hydrophilic, and has trivial immunogenicity.

The major problem facing the utilization of nanoparticles made from polymers as drug carriers is their elimination from the blood circulation rapidly via phagocytosis. Thus, the present interest has been directed towards the formulation of NPs coated with various macromolecules that can

| Parameters | Groups                  | MDA (nmol/mL) | TAC (U/ml) |
|------------|-------------------------|---------------|------------|
| Control group                        | 42.7±1.3         | 7.1±0.41     |
| Obese group                            | 86.4±3.9a        | 2.4±0.14a    |
| Ob+ Orlistat                           | 48.2±1.2b        | 5.9±0.12b    |
| Ob+ PLGA-CS-PEG nano-void              | 80.7±3.9c        | 2.9±0.21c    |
| Ob+ free Qu                            | 52.3±2.1b        | 4.9±0.18b    |
| Ob+ Qu NPs                             | 50.8±2.2b        | 5.3±0.27b    |
| Ob+ free CUR                           | 51.4±2.4b        | 5.1±0.29b    |
| Ob+ CUR NPs                            | 49.9±2.6b        | 5.4±0.31b    |

Data were depicted as Mean ± S.E for each group of rats n=8.
a: Change of significant at P<0.05 comparing with the control rats.
b: Change of significant at P<0.05 comparing with the obese rats.
c: Change of significant at P<0.05 comparing with the rats treated with orlistat.

| Parameters | Groups                  | TLR4 (pg/ml) | NF-kB (ng/L) | IL-10 (ng/L) |
|------------|-------------------------|--------------|--------------|--------------|
| Control group                        | 1458.8 ± 36.9     | 372.6± 20.4  | 281.4±13.3   |
| Obese group                            | 3167.6±237.8a     | 687.08±34.3a | 148.9±6.7a   |
| Ob + Orlistat                           | 1671.0±125.1b     | 434.98±9.8b  | 234.5±21.4b  |
| Ob + PLGA-CS-PEG nano-void              | 2970.7±52.0c      | 638.3±25.7c  | 168.9±7.6    |
| Ob + free Qu                            | 2052.47±65.5b     | 505.4±11.0b  | 191.1±15.8   |
| Ob + Qu NPs                             | 1948.1±87.9b      | 491.5±15.2b  | 208.7±18.3   |
| Ob + free CUR                           | 1970.9±72.2b      | 498.5±21.1b  | 201.3±14.6   |
| Ob + CUR NPs                            | 1897.3±154.9b     | 465.1±19.5b  | 210.2±16.8   |

Data were depicted as Mean ± S.E for each group of rats n=8.
a: Change of significant at P<0.05 comparing with the control rats.
b: Change of significant at P<0.05 comparing with the obese rats.
c: Change of significant at P<0.05 comparing with the rats treated with orlistat.
Fig. 3. Effect of orlistat, free Qu, Qu NPs, free CUR and CUR NPs on serum (a) LH and (b) E2 levels of obese rats

Data were depicted as Mean ± S.E for each group of rats n=8.

a: Change of significant at P<0.05 comparing with the control rats.
b: Change of significant at P<0.05 comparing with the obese rats.
c: Change of significant at P<0.05 comparing with the rats treated with orlistat.
restrain the process of phagocytosis and elevate of blood drug pharmacokinetics. Thus, in this scenario, we have originate Qu and CUR-loaded PLGA NPs, surface coated with PEG and chitosan to boost their therapeutic impact for management of obesity. The modulated, surface coated PEG NPs may avoid activity of macrophage uptake and prolong the encapsulated drug blood circulation time. Furthermore, chitosan coating inspires biocompatible, biodegradable and non-toxic system.

A reproducible and simple procedure has been adopted in the current study to fabricate and characterize Qu and CUR-loaded PLGA NPs coated with chitosan and PEG. The drug loaded nanocarriers characterization is physicochemically serve as a crucial merit for acquiring a potent drug delivery vehicle as it considerably monitors the fate of therapeutic carrier system at the site of action. In this situation, the size distribution is a remarkable parameter that promptly influences the drug release profile, physical stability, cellular uptake with in vivo bioavailability and biodistribution of the drug-loaded nanocargo. The dynamic light scattering findings in the current work, revealed that the developed Qu and CURNPs were of nanometer size range. Moreover, TEM images showed that the configuration of Qu NPs and CUR NPs contain core in which Qu or CUR were localized and surrounded by the coat of PLGA-CS-PEG. Furthermore, Qu NPs exhibited negative zeta potential of -8.5 mV, while, CUR NPs displayed positive zeta potential of +0.916 mV. Our findings regarding the characterization of the formulated Qu NPs were in accordance with those of Abd El-Fattah et al who cited that the prepared quercetin-loaded phyosome nanoparticles were homogeneously spherical with negatively charged zeta potential at -44.6 ± 4.1 mv. Besides, Tefas et al registered that the prepared samples of Qu-loaded PLGA NPs were negatively charged as a result of the ionized terminal carboxylic groups of PLGA exist on the surface of the NPs with zeta potential ranged between “27.5 ± 0.721 mV and “14.2 ± 1.069 mV. The obtained characterization results of the developed CUR NPs match those of Arya et al. These investigators found that the prepared CUR NPs were of size in nanometer range with an average diameter of 264 nm, a reduced polydispersity index (PDI) value of 0.181 and positive zeta potential of 19.1 mV. Furthermore, they demonstrated that the surface topology of CUR NPs (observed by atomic force microscopy (AFM) analysis) was smooth and spherical in nature.

The induction of obesity in rats by employment of HFD is considered as one of the most preferred and validated procedure to generate obese animal model for investigation of obesity and its mechanism, obesity-related complications, and screening of unprecedented medicinal interventions owing to its great relevance to the pathophysiology of obesity in human.

In this sense, our data revealed that rats fed the HFD for 12 weeks developed obesity characterized by an increase in body weight, body length, TC, AC, BMI and Lee index. Prior studies have shown that the differences in weight and lipid profile in blood can be used to ascertain the successful fulfillment of the obesity in animal model. Furthermore, Rocha et al stated that supplementation of rats with unsaturated HFD yields an increase in weight gain, total body fats and fat pads (retroperitoneal, visceral and epididymal). Also, the findings of Wang et al study clarified that, the weight of animals fed on HFD for 10 weeks was over 20 % greater than that of the control animals; the Lee’s index was significantly higher than that of the control animals; the waist circumference as well as visceral fat mass were also higher than those in the control one. Consequently, the obesity in the HFD fed animals could be allied to the elevated constitution of fat ingredient due to HFD regime.

The weight of the internal organs especially the fresh weight of the liver and fat, is important parameter for establishing HFD obesity. In this study, the untreated obese rats had a larger liver, right and left kidney, heart, spleen, adrenal, thymus and visceral, gonadal as well as subcutaneous adipose tissue weight in relation to the control rats. These findings echo those of Kabir et al who mentioned the increased liver weight in the HFD–fed animals than that of the control counterparts. Furthermore, they recorded the increased weight of mesenteric, peritoneal, and epididymal fats in the HFD–fed rats than that of the control one. Also, Tung et al registered the significant increase in the weight of kidney, liver, fat bodies around kidney and fat bodies around...
epididymis in the HFD-fed rats. Seo et al.\textsuperscript{57} cited that liver, kidney, spleen, thymus, epididymal and intestinal fat weights were increased in HFD group. Other investigators also stated that the weight of the heart\textsuperscript{58} and adrenal gland\textsuperscript{59} is increased in HFD group.

It has been proposed that mesenteric fat accumulation may lead to the lipid deposition in the liver owing to the immediate supply of free fatty acids via the portal vein\textsuperscript{60}. Moreover, obesity-linked inflammation and insulin resistance deteriorate the surplus free fatty acid metabolism in the liver and participate in the evolution of fatty liver which in turn increased the weight of the liver\textsuperscript{61}. Moreover, the kidney weight increment in obesity may be due to either compensatory hypertrophy of individual nephrons or due to accumulation of fluids and lipids\textsuperscript{62}. Furthermore, Leopoldo et al.\textsuperscript{58} found that obesity led to an increase in the interstitial collagen deposition in the left ventricle. Also, it was verified that a HFD for a 12-week period in rabbits causes fibrosis in coronary vessels, as well as assemblage of collagen in the cardiac interstitium\textsuperscript{63}.

The outcome of the present work demonstrated that the treatment of HFD-fed rats with orlistat, free Qu, Qu NPs, free CUR or CUR NPs caused a reduction in body weight, body length, TC, AC, BMI and Lee index. Furthermore, these treatments triggered a decrease in liver, right and left kidney, heart, spleen, adrenal, thymus and visceral, gonadal as well as subcutaneous adipose tissues weight. Karimi et al.\textsuperscript{64} represented that HFD rats treated with orlistat showed lower body weight, body length and BMI than untreated HFD rats. Also, these investigators found that the treatment with orlistat produced a decrease in fat mass, liver, kidney, spleen and heart weights. Furthermore, Othman et al.\textsuperscript{53} recorded that treatment of HFD rats with orlistat induced a decrease in Lee index and weight gain. Previous studies indicated that orlistat intake yields a reduction in body weight by 30% in obese population. The mode of action by which orlistat could reduce body weight is related to the decreasing of leptin level and adipocyte size\textsuperscript{65}.

The observed decrease in body weight upon treatment of HFD-fed rats with Qu is in congruity with the previous findings of Imessaoudene et al.\textsuperscript{66} and Perdicaro et al.\textsuperscript{67}. Imessaoudene et al.\textsuperscript{56} proved that the treatment with Qu induced a significant decrease in relative liver and adipose tissue weights in obese rats. Dong et al.\textsuperscript{52} reported that Qu has the ability to suppress fat accumulation in animal models. Furthermore, Rivera et al.\textsuperscript{68} pointed out that in obese Zucker rats, chronic administration of Qu markedly reduced body weight gain. Qu could reduce lipid aggregation in 3T3-L1 adipocytes\textsuperscript{69} and attenuate lipid cumulation via down-regulation of PPAR\(\alpha\) and C/EBP\(\alpha\). Also, Qu could induce adipocyte apoptosis via the inhibition of ERK1/2 phosphorylation and enhancement of the mitochondria pathway\textsuperscript{70}. In Western diet fed mice, Qu diminished fat cumulation in the liver and the expression of steatosis-linked genes\textsuperscript{71}. Accumulating evidence indicated that Qu acts by modulating hepatic cholesterol absorption and triglyceride assembly and production as well as via suppression of phosphodiesterase in both liver and adipose tissue\textsuperscript{68}.

Our data indicated that administration of CUR to HFD-fed rats lowered body weight gain and also the weights of the tested organs and tissues. These findings come in line with those of El-Wakf et al.\textsuperscript{72}. du Preez et al.\textsuperscript{73} recorded that CUR-PLGA NPs reduced liver fat deposition in HFD-fed rats. CUR has been found to inhibit adipose tissue angiogenesis, decrease preadipocytes differentiation and reduce lipids accumulation in adipocytes which in all aid in lowering body weight\textsuperscript{74}. du Preez et al.\textsuperscript{73} suggested that CUR-PLGA NPs reduces fat deposition through increasing energy expenditure. The underlying mechanisms by which CUR can decrease the accumulation of fat in adipocytes is the enhancement of AMPK, suppression of ACC enhancement, and down-regulation of the expression of lipogenic genes like SCD1, FAS and SREBP-1c\textsuperscript{89}. Also, CUR can boost lipolysis and motivate fatty acid ß-oxidation via up regulating hormone sensitive lipase (HSL), CPT-1, UCPs and PGC-1\(\alpha\). As well it can diminish the proliferation of pre-adipocytes, stimulate their apoptosis, and hearten cell cycle arrest. It also induces G0/G1 and G2/M cell cycle arrest and apoptosis of adipocytes\textsuperscript{9}. Additionally, CUR could down-regulate the expression of key genes implicated in adipocyte differentiation, such as C/EBP\(\alpha\), aP2 and PPAR\(\alpha\)\textsuperscript{75}.

In the current experiment, prolonged HFD feeding in yielded an impair in lipid metabolism, as manifested by greater levels of
Chol, TG, and LDL associated with lower levels of HDL. This observation is extremely supported by that of Noeman et al.76; Kabir et al.55 and Othman et al.53. HFD has been reported to trigger the synthesis of phospholipids and cholesterol esters in rats.77 In general, all lipids are absorbed into the blood from the gastrointestinal tract in the form of chylomicrons, composed of TGs, phospholipids, Chol and apolipoprotein B. The TGs in these chylomicrons are then digested as fatty acids and glycerol by lipoprotein lipase. These fatty acids are transported and stored in the liver and adipose tissues in the form of TGs. The remnants of the chylomicrons are fundamentally taken up by liver, and then transformed into several lipoproteins containing TGs, phospholipids, Chol and apolipoproteins. Among the lipoproteins, very low-density lipoproteins, composed of TGs, Chol and phospholipids, transport TGs that are formed in the liver to adipose tissues. LDL, which consists of Chol and phospholipids, transports Chol into peripheral tissues. Hence, elevated ingestion of lipids promotes the cumulation of body fat and increases Chol, phospholipids, and free fatty acids in the blood circulation that are transported to peripheral tissues. Furthermore, it has been hypothesized that elevated Chol and TG levels is the pivotal factor in lipoprotein metabolism, and their higher concentrations are ascribed to the elevated LDL formation and deposition, which is potentially atherogenic.78 The homeostasis of cellular Chol is preserved primarily via regulation of the LDL receptor and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which respectively influence the uptake and endogenous synthesis of Chol in the liver.79 Long-term consumption of HFD enhanced Chol biosynthesis and reduced expression of LDL receptor sites. Emerging data have proved that reactive oxygen species (ROS) interact with protein thiol molecules to generate a variety of sulfur oxidations which impaired the receptor signal of INS and hampers cellular uptake of TG from the blood circulation.81

Supplementation of obese rats with orlistat provoked a decline in serum Chol, TG and LDL levels along with a rise in serum HDL level. These results echo those of Karimi et al.84 and Othman et al.53. Carrière et al.82 reported that orlistat blocked dietary TGs hydrolysis and thus minimized the subsequent intestinal absorption of the lipolysis products monoglycerides and free fatty acids by covalently blocking the lipase active site. Furthermore, Bougoulia et al.83 reported the ability of orlistat to decrease serum LDL level because of its action towards suppression of gastric and pancreatic lipase enzymes.

The current data revealed that administration of obese rats with free Qu, Qu NPs, free CUR or CUR NPs lead to a decrease in serum Chol, TG and LDL levels in concomitant with an increase in serum HDL level. These findings match those of Seiva et al.84, Hu et al.85, Tong et al.86 and Shamsi-Goushkiet al.87. The hypolipidemic effects of Qu could be principally allied to its antioxidant properties88 and indirectly by affecting the Chol levels. Hence, LDL-Chol and VLDL-Chol would be protected against harmful impacts emerging from oxidative process.89 Other hypothesis includes the role of Qu in decreasing HMG-CoA reductase activity and consequently reducing Chol biosynthesis in the liver.88,89 Besides, there is growing evidence that Qu and its glycoside act by altering hepatic Chol absorption and TG assembly and secretion as well as via suppression of phosphodiesterases in the liver and adipose tissue.90

CUR is also recognized to have anti-hyperlipidemic activity by inhibiting the cumulation of TGs and Chol in the liver.92 The study of Shin et al.93 documented that CUR could inhibit the activity of hepatic HMG-CoA and down-regulate the gene of HMGR that codes for HMG-CoA enzyme in the liver. Rachmawati et al.92 recorded that the inhibition rate of HMG-CoA reductase by CUR nanoemulsion was greater than free CUR. Furthermore, CUR increases the transcription of liver X receptor alpha (LXRα), which regulates CYP7A1 (encoding cholesterol-7a-hydroxylase). Cholesterol-7a-hydroxylase plays role in the conversion of Chol into bile acids. In vitro using 3T3-L1 cells, it has been demonstrated that CUR can decrease the expression of glycerol-3-phosphate acyl transferase-1 (GPAT-1), a crucial enzyme for TG synthesis. In addition, CUR promotes adipocytes fatty acid ß-oxidation with a concomitant increase in the expression of carnitine palmitoyl transferase 1 (CPT-1), a key enzyme in transferring acyl-CoA into mitochondria for ß-oxidation.74 Also, CUR could suppress the
therosclerotic lesions formation in mice fed with atherogenic food, as indicated by the suppression of the atherogenic indicator and the rise in the HDL/total Chol percentage95. Importantly, it has been evidenced that nano-CUR is more effective than CUR in reducing the lipid profile97. This superior effect could be explained by the high sensitivity of CUR to the changes in physiological pH. In addition, CUR absorption through the gastrointestinal tract is very weak instead nano-curcumin has high bioavailability and uptake94.

In the current investigation, obese rats exhibited high serum glucose and INS levels as well as insulin resistance value. This result is comparable with the previous studies which clarified that rats fed a HFD display increased serum glucose concentrations61, 55. Besides, Xia et al95 documented that the feeding of rats with HFD for 8 weeks resulted in notably higher fasting glucose and fasting INS levels, as well as a higher HOMA-IR, which is indicative of increased insulin resistance compared with the control counterparts. These observations could be explained by the action of gram-negative bacterial cell wall contains lipopolysaccharide (LPS), which may transfuse the gut because of the leakiness of intestinal mucosa triggered by a high fat content in the diet96. Bacterial LPS may cause endotoxemia and inflammation97. Also, the intestinal permeability may ease the entry of bacterial fragments into the body which interact with the Toll-like receptor to enhance the innate and adaptive immunity and lead to hyperglycemia and insulin resistance98. Hsieh61 cited that some pro-inflammatory pathways interrupt INS signaling and play pathological roles in developing obesity-related type 2 diabetes mellitus. A growing body of evidence proposes that oxidative stress and inflammation cause insulin resistance via INS receptor substrate (IRS-1) serine/tyrosine phosphorylation instead of tyrosine/serine phosphorylation. Studies have shown that serine phosphorylation of IRS-1 attenuates the proximal INS signaling which results in insulin resistance99.

Treatment of obese rats with orlistat, free Qu, Qu NPs, free CUR or CUR NPs inspired a drop in serum glucose and INS levels as well as insulin resistance value. Karimi et al64 and Lee et al100 stated that serum glucose and INS levels are decreased in HFD rats treated with orlistat. The observed reduction in serum glucose and INS levels upon treatment of obese rats with Qu matches with the findings of Seiva et al84. Furthermore, Tong et al86 mentioned that Qu NPs decrease blood glucose level. Ying et al101 found that Qu is able to preserve pancreatic beta cell integrity. Qu consumption has been reported to significantly reduce the size of adipocyte, attenuate adipose inflammation, and prevent systemic insulin resistance. These impacts can be ascribed, in part, to the competence of Qu to motivate a healthy adipose tissue expansion, angiogenesis and adipogenesis97. In addition, the glycemic control could be improved by Qu via the inhibition of glucose absorption from intestine at the level of glucose transporters (GLUT), through enhancing the activity of glucokinase converting glucose into glucose-6-phosphate, a metabolite destined to glycogen synthesis. This action could be done via the increasing of the pancreatic islets number thus boosting INS release, and via metal chelating activity, herewith eliminating a causal factor for the generation of free radicals98.

The demonstrated drop in serum glucose level and insulin resistance value after treatment of obese rats with free CUR or CUR NPs is in parallel with the results of Shamsi-Goushkieiet al87. These actions of CUR and nano-CUR are possibly owing to the activation of PPAR-Y, motivation of glucokinase activity in the liver, induction of glucose transporter-4 (GLUT4) expression and attenuation of tumor necrosis factor-alpha (TNF-á)87. Na et al102 recorded that CUR improved INS sensitivity in muscle tissue of the rodent model of insulin resistant by an increase of the cellular glucose uptake as a result of the augmentation of GLUT4 translocation from intracellular compartments to the plasma membrane. In addition, increased glucose oxidation and glycogen synthesis have also been found in the C2C12 mouse skeletal muscle cells upon treatment with CUR103. In the study of Mantzorou et al. 104, the improvement of insulin resistance and the reduction of plasma glucose levels in diabetic rats have been demonstrated after treatment with CUR. Likewise, the study of Rahimi et al105 demonstrated that nano-CUR supplementation significantly reduced fasting blood glucose level compared to the placebo.

The obtained results in the current work demonstrated that obese rats experienced an elevation in serum LH level accompanied with a
reduction in serum E2 level. The observed increase in serum LH level and the decrease in serum E2 level are greatly supported by the recent findings of Yi et al. 106. Female fertility and reproductive health are compromised by overweight and obesity107. The prime factor underlying the adverse metabolic outcomes of obesity is thought to be insulin resistance. The decline in INS sensitivity affects not only glucose metabolism, but also all aspects of INS action108. INS has been reported to promote the proliferation of theca cells, the release of LH, and the number as well as affinity of LH receptors on granulosa cells109. Akamine et al108 stated the simultaneous occurrence of systemic insulin resistance and hyperinsulinemia together with the impairment of INS signaling in the ovary, and alteration of estrous cycle and of ovarian morphology in HFD-induced obesity in female rats. Insulin signaling has been involved in the regulation of female reproductive function by acting in both central nervous system and ovaries. The hyperinsulinemia can potentiate gonadotropin-stimulated steroidogenesis in granulosa and thecal cells, by elevating LDL receptor, 3α-hydroxysteroid dehydrogenase, 17α-hydroxylase, and 17,20 lyase expression110, 111. In addition, INS may act on the pituitary to boost LH release112. Gonadotropin-releasing hormone (GNRH) stimulates LH secretory response by a calcium-dependent signaling, and motivates mRNA and protein synthesis of LH via the MAPK signaling pathway. In the gonadotropes, both GNRH and INS stimulate the MAPK pathway, which is the point of signaling interaction and may be implicated with the potentiation of gonadotropin release by INS113. Furthermore, the cumulation of lipid droplets in the stromal and granulosa cells may be a consequence of hyperinsulinemia effect on the expression of the LDL receptor, which would contribute to enhance steroidogenesis108. Besides, it has been recorded that, insulin resistance and hyperinsulinemia prompt a serious imbalance in the synthesis of sexual steroid leading to excess androgen and slight estrogen levels and interfere with the effect of estrogen even at the receptor level114.

Treatment of obese rats with orlistat, free Qu, Qu NPs, free CUR or CUR NPs elicited a reduction in serum LH accompanied by an elevation in serum E2 level. Our data support the finding of Salehpour et al115 which indicated that the treatment of obese women with polycystic ovary syndrome (PCOS) with orlistat reduces the serum LH level through reducing body weight and improving hyperinsulinemia. The observed decline in serum LH level upon treatment of obese rats with Qu comes in line with the findings of Khorshidi et al116 who recorded that Qu supplementation decreases serum LH concentration in overweight or obese women with PCOS. It has been mentioned that resistin can promptly enhance steroidogenesis by elevation of LH receptor, steroidogenic acute regulatory protein, and the gene expression of INS receptor. Furthermore, resistin, along with LH, stimulates the synthesis of androgens via increasing INS receptor gene expression117. Khorshidi et al116 suggested that Qu can efficiently play a role in the modulation of serum LH through reducing resistin levels. On the other hand, the observed increase in serum E2 level in obese rats treated with Qu was found inconsistent with the observation of Nna et al118 which demonstrated that the improvement in serum concentration of E2 in the Qu-treated group is not far from the decrease in oxidative stress. Furthermore, Wang et al119 cited that the antioxidant capacity of the ovary is increased by administration of Qu via upregulating expression of some oxidative stress-related genes. Also, Qu is characterized by its phytoestrogen-like action120 and van der Woude et al. 121 stated that Qu had the ability to induce cell division in the MCF-7 cell line through the induction of ERα and ERβ expression. Neisy et al122 registered that Qu can enhance and increase uterine ERα gene expression. Our findings for diminished serum LH level and elevated serum E2 level after treatment of obese rats with CUR are consistent with previous studies123, 124. Various studies have established that CUR could stimulate the ovarian functions via its capability to enhance proliferation and reduce apoptosis125 while promoting folliculogenesis126 and steroidogenesis in murine ovarian cells127. A possible explanation for our findings is the ability of CUR to reduce insulin resistance128. Igbakin and Oloyede129 stated that CUR may include biomolecules that are capable to modify or stimulate INS receptors, alter glucose transport protein structure, and suppress INS antagonist in the body.

The consumption of a HFD has been found to promote oxidative stress which is apparent
in most experimental models and patients with clinical conditions. In the present setting, obese rats showed an elevation in the lipid peroxidation product; MDA level and a reduction in TAC. Our results are in accordance with the previous researches showing that tissue antioxidant defenses may be compromised in obese animals due to prolonged consumption of HFD. The imbalance between free radical production and antioxidant level results in oxidative stress, manifested by the diminished antioxidant defense system in the untreated obese rats in the current investigation. The depressed antioxidant defense system in obese rats results in damage to so-called lipid peroxidation. Yang and colleagues observed that the HFD-fed animals experienced high circulating concentration of thiobarbituric acid reactive substances, which are known as the indices of lipid peroxidation. The high caloric intake is a key factor in lowering fluidity of the mitochondrial membrane and enhancing ROS formation. Therefore, when ROS concentration is raised, the body's antioxidant defenses may be unable to cope leading to oxidative stress condition. Furthermore, it has been mentioned that, ROS stimulate inducible cyclooxygenase 2(COX-2) isoform and promote the biosynthesis of prostaglandin E, (PGE,), by activating extracellular signal–regulated protein kinase (ERK). It has been demonstrated that the up-regulated expression and activity of COX-2 isoform elevates the lipid peroxidation.

Increased oxidative stress leads to attenuation of lipoprotein lipase (LPL) activity; an enzyme implicated in the transformation of dietary lipids into sources of energy for peripheral tissues which leads to disturbance of lipid metabolism. The reduced activity LPL may be due to decreased expression of LPL and/or by an immediate effect of ROS on LPL. Yang and colleagues found that LPL activity is positively correlated with TAC. Thus, the observed decrease in antioxidant defense system in the current investigation could be the reason behind the impaired-lipid metabolism in HFD-fed rats due to the decreased activity of LPL. Also, previous studies have correlated both BMI and the oxidative stress status. Furthermore, impaired redox balance blights the secretion of INS, which can be considered as a cause of insulin resistance in obese individuals.

The data from this study demonstrated the prominence of the suggested treatment in rectifying the altered oxidant/anti-oxidant balance induced by prolonged consumption of HFD in rats. The recorded effect of orlistat in reducing serum MDA level and elevating TAC value comes in line with the findings of Othman et al. Yesilbursa et al. found remarkable decrease in MDA level in concomitant with orlistat-triggered weight loss. The improvement in the oxidative stress level might be ascribed to the ability of orlistat to prevent pro-atherogenic LDL Chol from oxidation. Also, Audikovszky et al. suggested that, the increase in HDL level by orlistat treatment not only protects the progress of atherosclerosis by reverse Chol transport, but also by its action on hydrolyzing lipid peroxidation product.

Plentiful of studies have indicated that Qu loaded NPs, had more anti-radical and antioxidative activities compared to free Qu. Encapsulating of Qu into PLGA-NPs promoted its activity as lipid oxidation inhibitor and its antioxidative properties. In addition, loading Qu into aminoalkyl methacrylate co-polymers)- poly vinyl alcohol NPs enhanced the anti-radical and antioxidant activities of Qu. In line with these studies, our data revealed that the efficacy of Qu NPs in restoring the oxidant/anti-oxidant balance in obese rats was superior than free Qu. Imessaoudene et al. confirmed that the treatment of obese rats with Qu decreases plasma MDA level and increased TAC. Furthermore, Al-Jameel and Abd El-Rahman registered that the treatment of diabetic rats with Qu NPs reduces the serum level of MDA and elevates the level of antioxidant enzymes. Polyphenols have been established as ROS scavengers and pro-oxidant metal chelators. Flavonoids antioxidant activities are based on their free-radical scavenging ability and their abilities to strongly chelate redox-active transition metal ions. The finding of Imessaoudene et al. demonstrated that the treatment with Qu alleviated oxidative stress in obese rats, by decreasing oxidant markers and elevating antioxidant defense molecules. This increment in antioxidants may be owing to neutralization of ROS as Qu has been recognized to scavenge superoxide anions and other free radicals in vitro. Qu is characterized by a strong scavenging activity of oxygen radicals and protection against lipid and protein oxidation which
has been mostly ascribed to its flavonoid fraction. Qu acts on the leading regulator of fat cumulation and metabolic disorders, particularly, by offsetting mitochondrial dysfunction and oxidative process.

Regarding the effect of treatment of obese rats with free CUR or CUR NPs on serum MDA and TAC value is compatible with the previous reports where CUR reversed the diminished total antioxidant status and attenuated the increase of the lipid peroxidation product, MDA. It has been reported that CUR displays its protective effect by modulating lipid peroxidation and boosting the antioxidant defense response. This action is attributed to the presence of the hydroxyl groups and methylene group of the â-diketone moiety. Prior reports have shown significant reduction of MDA and increased antioxidant enzyme activity in diabetic rats upon CUR treatment. Past studies recorded that CUR up-regulated nuclear factor erythroid-2-related factor-2 (Nrf2), a transcription factor that stimulates the expression of several genes, including those that encode for several antioxidant enzymes. He et al has cited that Nrf2 enhancement by CUR in HFD-fed mice thwarts muscular oxidative stress and mitochondrial redox imbalance.

Obesity usually triggers interruption in the immune function of the mature adipocytes, and motivates the release of several pro-inflammatory cytokines and chemokines while suppresses the anti-inflammatory cytokines. All these abnormalities result in the generation of obesity-associated chronic low-grade inflammation in adipose tissues that acts as the key to the initiation of different pro-inflammatory pathways.

Findings from this study showed that feeding of HFD, potentiates an elevation in serum TLR4 and NF-kB levels in concomitant with a decline in the serum IL-10 level. This finding is greatly supported by that of Zhang et al and Liu et al. TLR4 could regulate chemokine formation, neutrophil recruitment, and tissue damage. Former reports indicate that free fatty acid (FFA), especially saturated fatty acid, can activate TLR4-mediated pro-inflammatory signaling pathways. It has been also reported that chronic HFD intake led to TLR4-mediated RIP3 activation in mice and the knockdown of TLR4 down-regulates RIP3 expression and inflammatory response in palmitate-incubated hepatocytes. Furthermore, it has been found that TLR4 mediated signaling cascades in inflammatory diseases, such as activation of NF-kB and up-regulation of TNF-α. It has been registered that, TLR4 enhanced nuclear translocation of NF-kB by mediating the myeloid differentiation primary response protein (MyD88)-dependent early response pathway, thus initiating a series of inflammatory cascades by producing the pro-inflammatory factors and monocyte chemoattractants. Park et al. pointed out that NF-kB is a redox sensitive transcription factor that is activated by oxidative stress and its activation increases the expression of other pro-inflammatory mediators. IL-10 is recognized as one of the most remarkable anti-inflammatory and immunosuppressive cytokines that mainly acts on monocytes. It has been reported that there was a negative correlation between adipose IL-10 and serum TG. The effect of IL-10 on TG serum levels is attributable to enhanced hepatic FA synthesis and TG secretion. Therefore, dysregulation of IL-10 is directly or indirectly linked with the pathogenesis of obesity-related hypertriglyceridemia.

Treatment of obese rats with orlistat, free Qu, Qu NPs, free CUR or CUR NPs yielded a depletion in serum TLR4 and NF-kB associated with an elevation in the serum IL-10 level. The observed decrease in TLR-4 and NF-kB levels upon treatment of obese rats with orlistat is in harmony with the findings of El-Baz et al and Bansal et al. Also, the elevation in the serum IL-10 level in orlistat treated group is greatly supported by Li et al. findings. On the other hand, Perdicaro et al found that Qu supplementation attenuates HFD-induced TLR4 increase. Byun et al mentioned that Qu could suppress lipopolysaccharides-induced-TLR4 signaling in macrophages 264.7 through the positive regulation of Tollip expression (a negative regulator of TLR signaling). Furthermore, previous studies showed that Qu impedes NF-kB stimulation and thereby the generation of inflammatory mediators. Dias et al recorded that Qu decreases IкB-α degradation by inhibiting the upregulation of IκK-α and IκK-β, thereby prevented NF-kB activation. Xu et al stated that gold-Qu NPs can inhibit TLR4/NF-kB signaling in HFD-fed mice. The enhancement of serum IL-10 level in Qu treated rats is in concurrent with the findings of Ahmed et al.
Several studies have shown that CUR administration is implicated in the regulation of various inflammatory cytokines via inhibiting the activation of the TLR4/NF-κB signaling pathways\(^\text{179, 180}\). Feng et al.\(^\text{181}\) study revealed that CUR administration significantly down-regulated hepatic TLR4 and MyD88 expression, reduced p65 nuclear translocation and NF-κB DNA binding activity, indicating that CUR could suppress HFD-induced the activation of TLR4-MyD88/ NF-κB signaling in the liver, and then prevent liver fat accumulation induced by HFD in ApoE\(^{-/-}\) mice. Zhou et al.\(^\text{182}\) showed that CUR modulates macrophage polarization by inhibiting TLR4-MAPK/NF-κB signaling pathway. Wang et al.\(^\text{183}\) showed that CUR suppresses LPS-induced sepsis in mice via inhibiting TLR4 signaling activation. CUR was also found to exert an anti-inflammatory effect on rat vascular smooth muscle cells through suppressing ROS-related TLR4-MAPK/NF-κB signaling pathway\(^\text{184}\). CUR has been shown to inhibit the NF-κB pathway activity, which is mediated by the reduction of IkB kinase and p-IkB\(^\alpha\).\(^\text{185}\) Inhibition of NF-κB activity has been proposed as the most celebrated mechanism for the medicinal properties of CUR. Downregulation of NF-κB enhancement has been recorded to account for the blunting effect of CUR on inflammatory cytokine expression and release in both preadipocytes and differentiated 3T3-L1 adipocytes\(^\text{186}\). Yang et al.\(^\text{187}\) found that CUR-loaded chitosan-bovine serum albumin NPs induces the lower protein expression levels of TLR4 than free CUR, with concomitant inhibition of NF-κB phosphorylation. These investigators suggested that CUR-loaded chitosan-bovine serum albumin NPs may motivate CUR-induced macrophage phagocytosis by suppressing M1 macrophage polarization via hindering the TLR4-MAPK/NF-κB signaling pathway, thus boosting curcumin’s anti-inflammatory effect. Coinciding with our finding, Mollahazadeh et al.\(^\text{188}\) cited that CUR can enhance the production of anti-inflammatory IL-10 to counteract inflammatory mediators.

Conclusively, the data presented herein highlighted the prominent role of Qu and CUR in combating obesity and its related metabolic alterations via their hypolipidemic, hypoglycemic, antioxidative and anti-inflammatory mechanisms. Interestingly, both Qu and CUR NPs showed a preferable effect than their free counterparts probably due to increased solubility and bioavailability of nano-formulations. The promising therapeutic effect of Qu and CUR modified nanoencapsulation against obesity may have a suitable impact on achieving clinically convenient approaches to treat patients with obesity in the future.

ACKNOWLEDGMENTS

None.

Conflict of interest

The authors declare that there is no any conflict of interest

Funding

No funding source.

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