Original Article

Protective and Antioxidant Effects of Quercetin Loaded Black Cumin (*Nigella sativa L*) Seed Oil-Based Nanoemulsion in Testosterone-Induced Benign Prostatic Hyperplasia: An Experimental Study

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How to cite this article: **Jafari A, Panahi N, Hesaraki S, Akbari G. Protective and Antioxidant Effects of Quercetin Loaded Black Cumin (*Nigella sativa L*) Seed Oil-Based Nanoemulsion in Testosterone-Induced Benign Prostatic Hyperplasia: An Experimental Study. Archives of Razi Institute. 2024;79(5):1065-1074.** DOI: 10.32592/ARI.2024.79.5.1065

Article Info:
Received: 25 November 2023
Accepted: 16 March 2024
Published: 31 October 2024

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ABSTRACT

Quercetin (Qu) is a type of plant flavonoid that has been demonstrated to possess anti-proliferative properties, making it a potentially beneficial agent in the treatment of prostate hyperplasia. Additionally, Nigella sativa seed oil (NSO) has demonstrated efficacy in alleviating symptoms associated with benign prostatic hyperplasia (BPH). The objective of this study is to evaluate the impact of quercetin (Qu) in combination with a nanoemulsion derived from Nigella sativa seed oil (Qu-NSO) on a rat model of benign prostatic hyperplasia (BPH). The study employed a rat model of BPH, whereby testosterone enanthate (5 mg/kg) was administered subcutaneously to induce the condition. The rats were then treated with various interventions, including Qu (25 mg/kg and 50 mg/kg), NSO, and Qu-NSO (25 mg/kg and 50 mg/kg), with a total volume of 0.5 ml administered orally. This allowed for an assessment of the effects of Qu-NSO. The self-nanoemulsified drug delivery system was prepared using NSO, coconut oil, Tween 80, and polyethylene glycol 400 (PEG). The globule size and zeta potential of the formed vesicles were determined. Investigations were conducted on the rat model to examine the effects of the treatments on dihydrotestosterone (DHT), prostate-specific antigen (PSA), prostatic weight and index, oxidant and antioxidant markers, and histopathology. The mean globule size for Qu-NSO was 171.9±10.9 nm, with a zeta potential value of +17.3 mV. The Qu-NSO treatment resulted in a 40% reduction in prostate weight and an 86.71% reduction in prostate index compared to the testosterone-treated group. The Qu-NSO treatment resulted in a significant reduction in serum levels of oxidative contents (MDA) ($p<0.0001$), while antioxidative substances (SOD and GPx activity) exhibited a significant increase ($p<0.0001$). The Qu-NSO group exhibited superior outcomes in terms of decreasing prostatic weight and enhancing antioxidative properties, as evidenced by elevated antioxidant enzyme activity. This study demonstrated that the Qu and NSO in a Qu NSO formula augmented the Qu efficacy in managing BPH. Quercetin (Qu) is a type of plant flavonoid that is beneficial in fighting prostate hyperplasia cells. Meanwhile, *Nigella sativa* seed oil (NSO) has shown promise in relieving benign prostatic hyperplasia (BPH) symptoms. This study aims to assess the effects of Qu combined with NSO-based nanoemulsion (Qu-NSO) against a rat model of BPH. The study involved the induction of BPH in rats using testosterone enanthate (5 mg/kg) subcutaneously and administering different treatments, including Qu (25 mg/kg and 50 mg/kg), NSO, and Qu-NSO (25 mg/kg and 50 mg/kg), with total volume 0.5 ml per oral to assess the effects of Qu-NSO. NSO, coconut oil, Tween 80, and polyethylene glycol 400 (PEG) were obtained for the self-nano-emulsified drug delivery system. The globule size and zeta potential of formed vesicles were measured. Dihydrotestosterone (DHT), prostate-specific antigen (PSA), prostatic weight and index, oxidant and antioxidant markers, and histopathology were investigated in the rat model. The average globule size for Qu-NSO was 171.9±10.9 nm, with a zeta potential value of +17.3 mV. Qu-NSO declined prostate weight by 40% and prostate index by 86.71% compared to the testosterone group. Qu-NSO treatment significantly reduced the serum levels of oxidative contents (MDA) ($p<0.0001$), while antioxidative substances (SOD and GPx activity) were significantly more ($p<0.0001$). Qu-NSO was superior to the finasteride group in decreasing prostatic weight and antioxidative properties, such as increasing antioxidant enzyme activity. This study revealed that the Qu and NSO in a Qu NSO formula enhanced the Qu efficacy in managing BPH.

Keywords: Prostate Enlargement, Noncarcinogenic Behavior, Alternative Medicine, Hyperplasia, Self-Nano-Emulsified Drug Delivery System.

1. Introduction

Benign prostatic hyperplasia (BPH) exerts pressure on the urethra and increases the thickness of the bladder wall, leading to urine retention (1). An increase in dihydrotestosterone (the active form of testosterone, DHT) and an imbalance in estrogen and testosterone levels are responsible for the development of hyperplasia and prostate enlargement. The principal pharmaceutical agents employed for the management of benign prostatic hyperplasia (BPH) encompass tamsulosin (an alpha-1 receptor inhibitor), sildenafil, and tadalafil (phosphodiesterase type 5 inhibitors), which induce relaxation of prostatic smooth muscle, and finasteride (5α-reductase inhibitors), which impedes the synthesis of dihydrotestosterone (DHT) (2). Although these drugs have been demonstrated to be effective, the adverse effects, which include impotence, orthostatic hypotension, and gynecomastia, have prompted an increased focus on the exploration of alternative methods for the management of BPH. The most extensively studied flavonoid in vegetables and fruits, quercetin (3,5,7,3′,4′-pentahydroxyflavone, Qu), has been demonstrated to possess a multitude of beneficial properties, including antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, and antihypertensive effects, as well as anti-atherogenic activity (4, 5). Recent studies have indicated that quercetin (Qu) may reduce testosterone levels, luteinizing hormone, and estradiol (6) and potentially decrease the levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in the blood (7). Additionally, quercetin is employed as a dietary supplement and therapeutic agent for prostate hyperplasia due to its mechanism of action, which encompasses the induction of apoptosis, the inhibition of androgen receptors, the inactivation of the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway, and the prevention of angiogenesis (8). Furthermore, PI3K/AKT plays a pivotal role in the proliferation and progression of prostate hyperplasia and facilitates the prevention of apoptosis (9). Consequently, drugs based on quercetin are of significant interest in the treatment and prevention of benign prostatic hyperplasia (BPH). However, quercetin has several limitations due to its poor water solubility, low chemical stability, and low bioavailability (11). It is therefore imperative to conduct trials with the objective of enhancing the water solubility of quercetin, its bioaccessibility, and chemical stability in water, as well as confirming its delivery and absorption. NSO has a long history of use in the treatment of a number of conditions, including influenza, asthma, bronchitis, cough, dizziness, hypertension, fever, inflammation, headache, and eczema. Additionally, black seed oil has been investigated for potential therapeutic applications in various conditions, including diabetes, hypertension, immunomodulatory and anti-inflammatory drugs, neuroprotective agents, and cancer. Moreover, nutraceuticals, particularly black cumin, represent a promising avenue for therapeutic intervention in prostate and bladder conditions. The seeds are a rich source of fixed oil, comprising a high proportion of unsaturated fatty acids, including oleic, linoleic, and linolenic acid. They have been demonstrated to prevent the proliferation of prostate cells induced by DHT and testosterone (12, 13). Additionally, NSO has been demonstrated to inhibit the 5-α-reductase enzyme, which is a drug target for DHT and is responsible for converting testosterone into DHT (12). Thymoquinone, the primary bioactive component of NSO, has been frequently employed for the treatment of migraines and a range of other ailments, including obesity, asthma, gastrointestinal disorders, menstrual issues, and lactation (13). Nevertheless, there is little research examining NSOs engaged in in vivo experiments and purported to have a beneficial impact on the prevention of testosterone-induced BPH (12-14). Self-nano-emulsified drug delivery systems are increasingly being utilized in various formulations within the pharmaceutical field as vehicles or drug delivery systems due to their high degree of drug penetration through tissues, stability, and fewer adverse effects associated with other novel drug delivery systems (11, 13, 17). Nanoemulsions are superior to microemulsions due to their smaller size, greater surface area, and the absence of characteristic creaming, flocculation, coalescence, or precipitation (18). Nanoemulsions are composed of isotopic combinations of oil, surfactants, and co-surfactant constituents that spontaneously form emulsions when in contact with gastrointestinal fluid (19). In addition to enhancing stability, these lipid-based nanoparticles, when employed as novel drug delivery systems (DDSs), have been demonstrated to facilitate the solubility and dissolution behavior of drugs within the gastrointestinal tract (GIT), thereby enhancing the absorption of poorly water-soluble drugs (15, 16). One of the key objectives of nanomedicine is to enhance the efficacy of active pharmaceutical agents for the treatment of chronic disorders. Consequently, Qu was incorporated into an NSO-based nanocarrier. The objective of this study was to evaluate the efficacy of a Qu-loaded NSO self-nanoemulsified drug delivery system (Qu-NSO SNEDDS) formulation in the management of benign prostatic hyperplasia (BPH).

2. Materials and Methods

2.1. *Nigella Sativa* Oil

The crude NSO and virgin coconut oil were procured from the Research Institute of Medicinal Plants and Raw Materials, Faculty of Pharmacy, Shahid Beheshti University. All chemicals, including Qu, polyethylene glycol (PEG) 400, and Tween 80, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Testosterone enanthate (100 mg/mL) ampoules were provided by the Chemical Industries Abu Rayhan (Iran).

2.2. Qu-NSO Formulation

A homogeneous mixture was formed by stirring a solution comprising 3% Qu, 30% Tween 80 (the surfactant), 10% coconut oil, and 10% NSO for 10 minutes at 50°C (15, 16). A solution of PEG 400 (20%) in deionized water (27%)

was added to the oil phase in small quantities at 50°C. To create a nanoemulsion with a concentration of 1.5% Qu, the previous steps should be repeated, with the addition of 28.5% deionized water. Following a period of cooling at room temperature, the mixture was subjected to ultrasonication using a probe sonicator (20 kHz, 1500 W, Sonics, Newtown, CT, USA) for a duration of 10 minutes. Subsequently, a self-nanoemulsified drug delivery system was formed and stored at 4°C (20).

2.3. Qu-NSO Assessment

The predisposition of Q-NSO aqueous dispersions to emulsify spontaneously and with clarity was evaluated. The stability of the samples was evaluated through three distinct steps. The initial step, centrifugation, entailed diluting the formulation with an aqueous medium prior to centrifuging the mixture at 15,000 rpm for 15 minutes, after which the resulting combination was observed for phase separation. Subsequently, the second and third stages entailed subjecting the sample to freeze-thaw and heating-cooling cycles, respectively. Initially, the mixture was diluted with deionized water in a 1:50 ratio. Subsequently, the mixture was maintained at a temperature of -20°C for a period of two days, after which it was permitted to melt and observed for evidence of phase separation. Subsequently, the mixture was maintained at 40°C for a period of two days, after which it was allowed to cool and examined for phase separation.

2.4. Vesicular Size and Zeta Potential

The vesicular size, zeta potential, and mean size of the nanoemulsion of *Nigella sativa* seed oil were assessed by Nano-ZS SZ-100 (Horiba, Japan) using the dynamic light scattering (DLS) technique.

2.5. Qu-NSO examination by Scanning Electron Microscopy (SEM)

The external morphology of the particles was evaluated through observation under a scanning electron microscope (SEM) using a TESCAN-Vega 3 (TESCAN SEM, Czech Republic).

2.6. Qu-NSO Formulation Experimental Studies

Ten-week-old male Wistar rats (weight, 180-200 g) were provided with ad libitum access to food and maintained in an air-conditioned room (22±2°C, 55±5% humidity) under a 12-hour light/12-hour dark cycle. The current study was approved by the Biomedical Research Ethics Committee of the Islamic Azad University—Science and Research Branch (approval ID: IR.IAU.SRB.REC.1399.179). The animals were allowed to acclimatize for a period of one week prior to the commencement of the experiments. In this study, a total of 42 rats were randomly assigned to seven experimental groups, with six rats per group. The BPH model was induced by the subcutaneous (SC) administration of testosterone enanthate (5 mg/kg) at a daily dose for a period of four weeks. Group T was administered testosterone in a dosage of 5 mg per kilogram of body weight, administered subcutaneously. Group T+F was administered finasteride (1 mg/kg orally) in conjunction with testosterone (5 mg/kg, subcutaneous). Group T+NSO was treated with a black cumin nanoemulsion (NSO, 1 ml, orally) in conjunction with testosterone (5 mg/kg, SC). The T+Qu25 and T+Qu50 groups were administered a low and high dose of Qu, respectively, orally in conjunction with testosterone (5 mg/kg, SC). The low dose of Qu was 25 mg/kg, while the high dose was 50 mg/kg. The T+Qu25-NSO and T+Qu50-NSO groups were administered Qu-loaded NSO-based nanoemulsion (Qu-NSO, 1 ml) orally, in conjunction with testosterone (5 mg/kg, SC), at low (25 mg/kg) and high (50 mg/kg) doses of Qu-NSO for four weeks. Carbon dioxide was employed as the euthanasia agent following a period of 72 hours following the administration of the final doses.

2.7. Prostate Index and Serum Evaluation

The prostatic index (defined as the ratio of prostate weight to body weight, expressed in milligrams per gram) was calculated. The serum was obtained for the purpose of measuring dihydrotestosterone (DHT), prostatic-specific antigen (PSA), and oxidative stress parameters, including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities (21). All procedures were conducted in accordance with the instructions provided by the manufacturer of the ZellBio Kit (GmbH).

2.8. Prostate Tissue Evaluation

The three prostate lobes (anterior, dorsal, and lateral) were collected and the length, width, and weight were determined. The specimens were then subjected to hematoxylin and eosin (H&E) staining. The size and thickness of epithelial cells in the prostate tissue were assessed to identify the Qu-NSO effect on BPH. This was done by assigning character scores of 0 (normal), 1 (slight increase), 2 (significant rise), and 3 (intense increase). The remaining tissues were held at -70°C for analysis.

2.9. Statistical Analysis

The data were subjected to analysis using one-way ANOVA, statistical tests (One Way ANOVA, Kruskal-Wallis), and the paired T-test. The preparation of graphs was conducted using the appropriate software (GraphPad Prism) with SPSS version 25 software. For all comparisons, a significance level of $p < 0.05$ was considered.

3. Results

3.1. Evaluation of Qu-NSO Formulation

3.1.1. Globule Size and Zeta Potential

The combination of coconut oil (10%), NSO (10%), tween (30%), and PEG 400 (20%) was found to result in a globule size of 171.9±10.9 nm, as indicated by the mixture designs (Figure 1). The mean zeta potential of the mixture was +17.3 mV (Figure 2).

3.1.2. Surface Examination by SEM

The scanning electron microscopy (SEM) images demonstrated that the formed vesicles exhibited a spherical morphology. The investigation of the Qu-NSO revealed that the average particle size of the Qu-NSO was 1000 nm in diameter. Nevertheless, the SEM (20,000x) analysis revealed that the particles were clustered and exhibited the

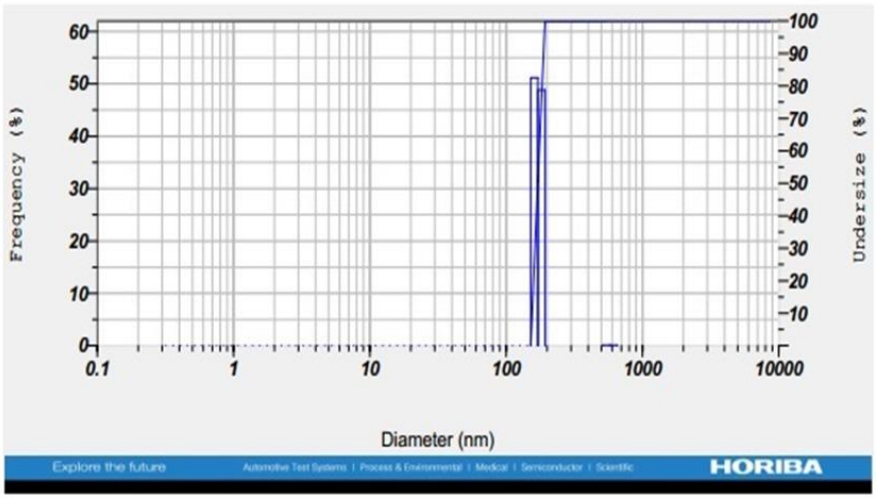

Figure 1. Globule size (nm) of black cumin seed oil nanoemulsion.

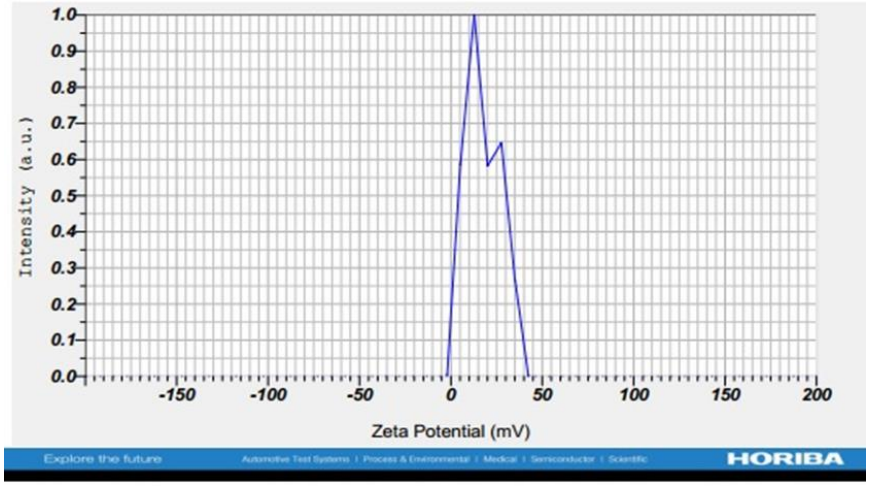

Figure 2. Zeta potential (22) of nanoemulsion with Electrophoretic Mobility Mean: 0.000135 cm /Vs.

appearance of minute beads within Qu-NSO groups (Figure 3). Particles with a size between 100 and 1000 nm are deemed suitable for micro-nano grade particles. Moreover, the SEM analysis demonstrated that the particle size was larger than that observed in the DLS, which is likely attributed to the presence of Qu in the nanoemulsion.

3.2.1. Effect of Qu-NSO on body weight, prostatic weight, prostatic index, prostate-specific antigen, and dihydrotestosterone

The results demonstrate a statistically significant increase in body weight, prostate weight, prostate index, and DHT in the T group. Finasteride was observed to effectively reduce prostate weight, prostatic index, PSA, and DHT levels ($p<0.001$). No significant change was observed in body weight when compared to the control group. The low-dose Qu nanoemulsion resulted in a statistically significant reduction in prostate weight (9%), prostate index (46%), and DHT (61%) compared to the control group. The administration of Qu nanoemulsion at a dose of 25 mg/kg/day resulted in a statistically significant reduction in body weight, with a mean decrease of 25%. The combination of Qu (25 mg/kg) and black seed nanoemulsion demonstrated a notable divergence in prostate index and DHT levels when compared to the F+T group. It is noteworthy that a substantial decrease in PSA was only observed when nanoemulsion was utilized as the drug delivery method (Table 1).

3.3. Effect of Qu-NSO on the Prostatic Tissue

A hyperplastic change was observed in the histological architecture of prostatic tissue in animals that received T (5 mg/kg) subcutaneously for 28 days. The presence of papillary hyperplasia was indicated by the observation of alveolar folding with the presence of tall epithelial cells (Figures 4 and 5a). The alveolar cells were tall columnar and exhibited minimal hyperplasia in a papillary form in the T+F group (Figure 5b). The lumina of the prostate glands in rats treated with finasteride exhibited a reduction in size when compared to the T group (Figure 5b and Figure 5a).

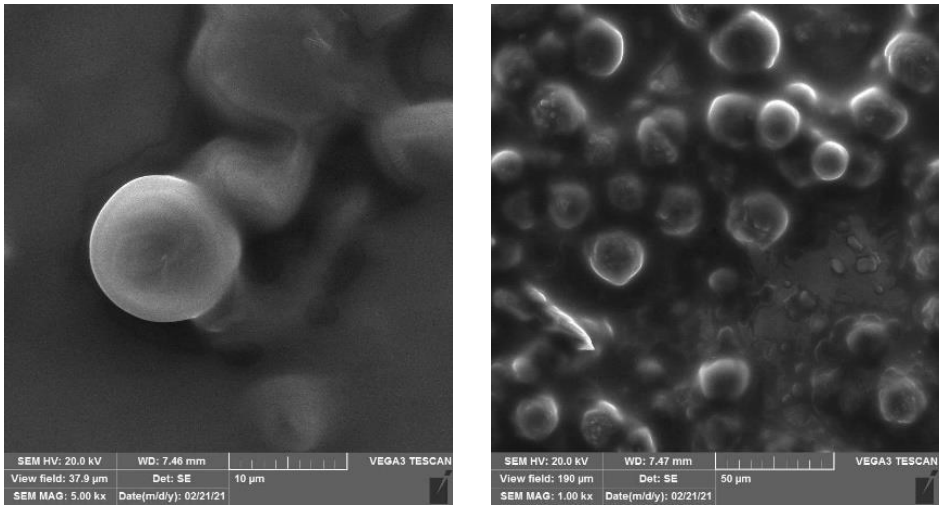

Figure 3. SEM images of Qu-NSO

Table 1. Effect of Qu-NSO on Body Weight (BW), Prostatic Weight (PW), Prostatic index (PI), prostate-specific antigen (PSA), and dihydrotestosterone (DHT) of testosterone-induced BPH rat model.

groups	T	T+F	T+NSO	T+Qu25	T+Qu50	T+Qu25NSO	T+Qu50NSO
BW (g)	257.5±12.6	263.7±8.5	255±10.8	252.5±10.4	253.7±12.5	235±12.9*	242.5±6.45
PW (g)	2.598±0.07	1.6±0.08***	1.57±0.05**	1.65±0.05**	1.6±0.045**	1.4±0.02***	1.57±0.02**
PI	10.06±0.3	6.16±0.3***	6.07±0.3***	6.59±0.3**	6.06±0.2***	5.3±0.2$^{****\#}$	6.03±0.05***
PSA (ng/ml)	0.7±0.01	0.34±0.02**	0.44±0.02**	0.63±0.02$^{\#}$	0.5±0.02$^{**\#\#}$	0.33±0.03***	0.51±0.02$^{***\#\#}$
DHT (ng/ml)	3.6±.05	2.96±0.01**	1.67±.04***	2.8±0.03**	2.2±0.04*	1.4±0.03$^{**\#\#\#}$	1.6±0.04$^{**\#\#}$

PI = prostate weight (mg) / body weight (g). T: testosterone enanthate (5 mg/kg, SC). F: finasteride (1 mg/kg, orally), NSO: *Nigella sativa* oil nanoemulsion (1 ml, orally), Qu25 and Qu50: Quercetin (25 mg/kg and 50 mg/kg), Qu25NSO and Qu50NSO: Qu-loaded NSO (25 mg/kg and 50 mg/kg), Values are the means ± SE (n = 6). Statistical significance: *$\rho < 0.05$ and **$\rho < 0.01$ compared with the T group; # $\rho < 0.05$ and ## $\rho < 0.01$ compared with the F+T control group. Statistical analysis was determined using Tukey's test

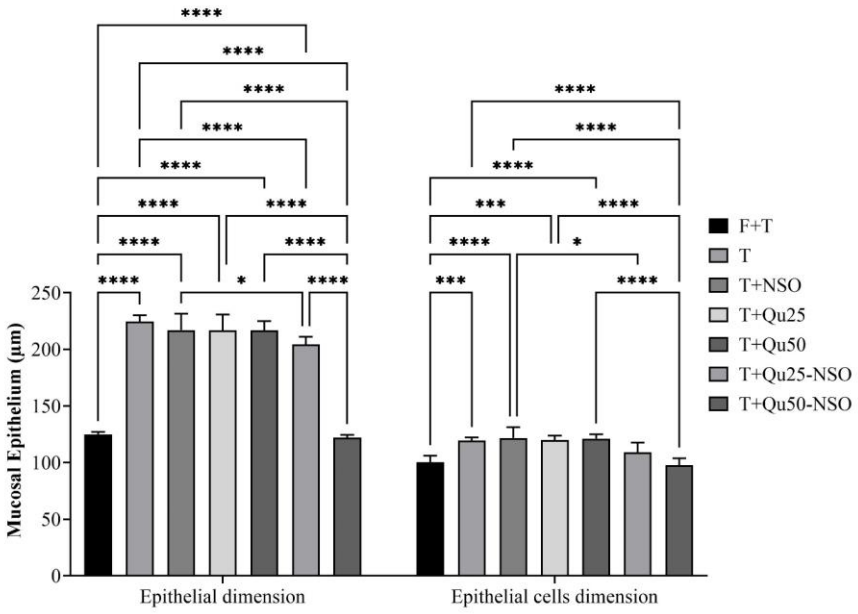

Figure 2. Effects of nanoemulsion of black seed oil containing Qu, finasteride, and nanoemulsion of black seed oil on epithelial cell diameters in the BPH model.

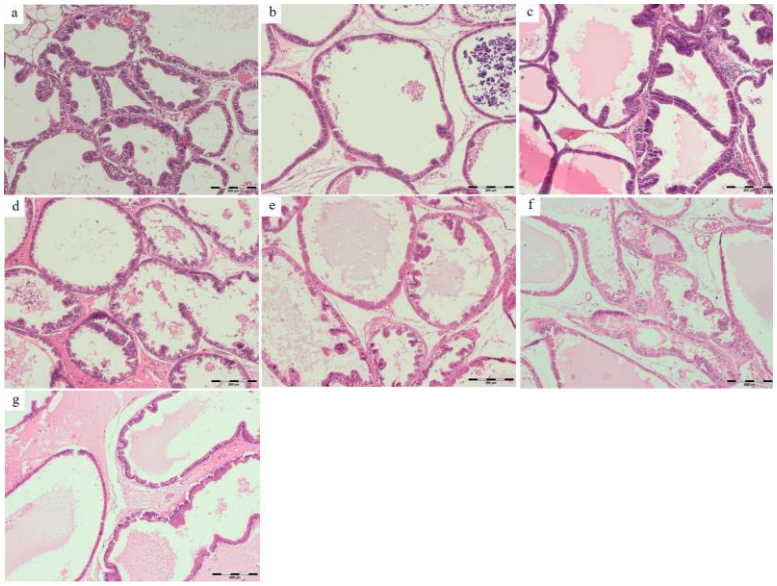

Figure 3. Histopathological characteristic aspect in prostate tissue (H&E staining, 200×)—arrow: Papillary projection. Scale bar=100 µm. Effects of nanoemulsion of black seed oil containing Qu, finasteride, and nanoemulsion of black seed oil on BPH. (a) the T group received testosterone (5 mg/kg, SC), (b) the T+F group received finasteride (1 mg/kg), (c) the T+NSO group received nanoemulsion of black seed oil, (d and e) T+Qu25 and T+Qu50 groups received Qu (25 and 50 mg/kg, daily), (f and g) T+Qu25NSO and T+Qu50NSO groups received nanoemulsion of black seed oil containing Qu (25 and 50 mg/kg, daily).

The papillary growth with a tall epithelial lining remained hyperplastic, exhibiting a level comparable to that observed in the T group in the T+Q25 (Figures 4 and 5c) and T+Qu50 (Figures 4 and 5d) groups. The tall hypertrophic epithelium and papillary hyperplasia observed in the T group showed improvement, resulting in a reduction in the number of papillary projections and cells with a histological architecture that was nearly normal in the T+Qu25NSO (Figures 4 and 5e) and T+Qu50NSO (Figures 4 and 5f) groups. The lowest alveolar epithelium and intraluminal folding were observed in the T+F and T+Qu50NSO groups (Figure 5G).

3.4. Effect of Qu-NSO on the Serum MDA, SOD, and GPx

The mean serum malondialdehyde (MDA) concentration was significantly elevated in the T+F groups relative to the T group (mean difference: 28.33 nmol/L serum, $p<0.05$).

The administration of Qu-NSO at both low and high doses led to a notable reduction in serum MDA activity when compared to the T group (mean difference: 38.89 nmol/L serum, $p<0.05$) and the F+T group (mean difference: 67.22 nmol/L serum, $p = 0.0045$) (Figure 6A). Furthermore, the mean serum SOD activity exhibited a marked decline in the T+Qu25-NSO group when contrasted with the T group (mean difference: 45.70 U/ml, $p = 0.0004$). The Qu-NSO treatment resulted in a notable elevation in SOD activity when compared to the T+Qu group (mean difference: 22.68 U/ml, $p < 0.0095$) (Figure 6B). Additionally, a statistically significant distinction was observed in GPx levels between the Qu-NSO and T ($p < 0.0001$), F+T ($p < 0.0001$), T+Qu25 ($p < 0.0001$), and T+Qu50 ($p < 0.0001$) groups. No significant difference was observed in SOD activity between the T+Qu25-NSO and T+Qu50-NSO groups (Figure 6C).

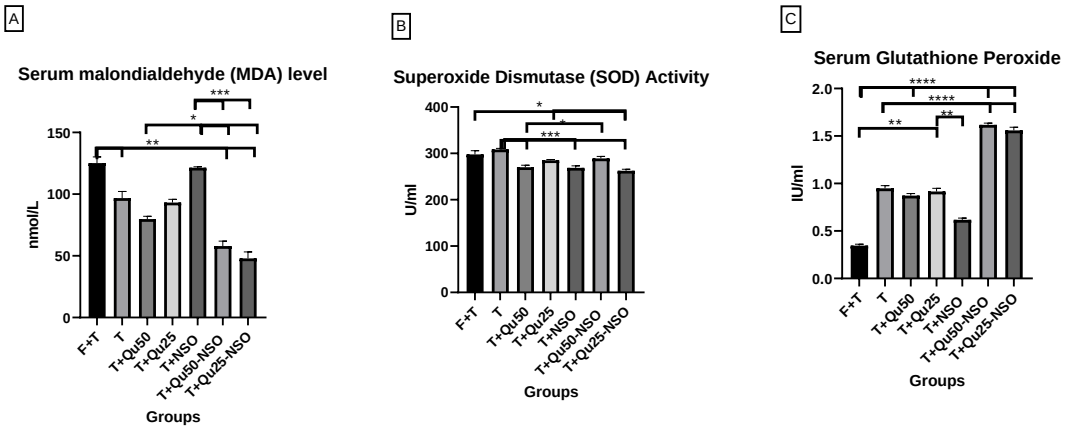

Figure.4. Effect of Qu-NSO SNEDDS on the Serum MDA (A), SOD (B), and GPx (C): all rats were treated for 28 days testosterone (T, 5mg/kg, SC), T+F group received finasteride (1 mg/kg), T+Qu25 and T+Qu50 groups received Quercetin (25 and 50 mg/kg body weight daily), T+Qu25NSO and T+Qu50NSO groups received nanoemulsion of black seed oil containing Quercetin (25 and 50 mg/kg) and (f) T+NSO group received nanoemulsion of black seed oil.

4. Discussion

The primary objective of the combination distribution of Qu with NSO was to augment the biological activity of Qu against BPH. The rationale behind the use of NSO, with its well-documented activity in prostate tissues, as the oil constituent for NSO, is based on this established activity. The Qu-NSO formulation was developed through an experimental design with the objective of enhancing efficacy by controlling the nanoemulsion size and charge. The nanoemulsion for the delivery of Qu exhibited optimal thermal storage stability and high bioaccessibility (11). The findings of Kumar et al. (23) indicate that Qu nanoparticles are more stable and efficacious than free Qu, due to the controlled release of Qu from the polymer matrix (nanoparticles). The potential mechanism underlying the enhanced biological activity observed following the loading of Qu in polymeric nanoparticles can be attributed to alterations in membrane permeability, adsorption, and the formation of bonds between the nanoparticles and the cell membrane (18). The animals in the experimental groups did not exhibit any signs of toxicity and were observed to survive. It has been demonstrated that doses of Qu-loaded NSO can effectively reduce the proliferation of prostate cells, prostatic weight, and overall body weight. The findings of this study indicate that the combination of quercetin and NSO in the Qu-NSO formula resulted in enhanced efficacy of quercetin in the management of benign prostatic hyperplasia (BPH). The reduction in DHT levels was only observable when Qu-NSO (25 mg/kg) was employed. The results of human studies indicated a reduction in serum DHT levels following Qu utilization. Nevertheless, it has been extensively documented that the effects of Qu on the androgenic system are primarily mediated by the modulation of the androgen receptor (AR) rather than the production of DHT (24). It is established that increasing the bioavailability of quercetin (Qu) in the tumor microenvironment results in a significant inhibitory effect on the androgen receptor (AR) gene and its downstream targets, with a dose-dependent response. Chronic inflammation induced by testosterone in prostatic epithelial cells results in gradual cell hyperplasia, which in turn causes urinary retention, frequent urination, urinary tract infections, and the formation of bladder stones (25). The objective of this study was to enhance the delivery of quercetin and to evaluate the combined effects of quercetin-NSO against a rat model of benign prostatic hyperplasia (BPH). A testosterone-induced BPH rat model is a commonly utilized model for the evaluation of potential anti-BPH agents (26). The induction of BPH in these models is characterised by an increase in prostate weight, size, volume, and index, as well as elevated levels of DHT. Pathological BPH markers include glandular cavity enlargement, prostatic epithelium and stromal cell proliferation, and inflammatory cell infiltration (27). The results of this study demonstrated that treatment of testosterone-induced BPH in rats with Qu-NSO for 28 days resulted in a significant reduction in BPH progression, as evidenced by a decrease in prostate weight, prostate index, and histopathological severity. Furthermore, treatment with Qu-NSO at 25 and 50 mg/kg and 5 mg/kg of finasteride resulted in elevated testosterone levels in the rat serum and prostatic tissue. This treatment confirmed the in vivo inhibitory efficacy of Qu-NSO against 5α-reductase, and the observed effects followed a dose-dependent pattern. The nanoemulsion drug delivery method has been shown to increase the effective drug dose, thereby lowering the prostate index to a greater extent. Qu is a prominent dietary flavanol, commonly found in fruits and vegetables. Its anti-hyperplasia effects involve the induction of cell viability loss, apoptosis, and autophagy through the modulation of pathways such as PI3K/Akt/mTOR, Wnt/β-catenin, and MAPK/ERK1/2. Additionally, qu has demonstrated potential against BPH by enhancing EER-ɣ transcriptional activity, exerting an anti-androgenic effect, and exhibiting selective androgen modulator activities (28-30). In a series of studies, Fu and colleagues demonstrated that Tonglong qibi was effective in alleviating benign prostatic hyperplasia (BPH) in rats without causing any adverse effects on the liver or reproductive system. It was determined that Qu is the essential element that facilitates the treatment of BPH with Tonglong qibi. The compound demonstrated the ability to reduce proliferation and oxidative stress while increasing Nrf2 expression in hyperplastic prostate epithelial cells, indicating that Qu in Tonglong qibi exerts its beneficial effects on BPH by inhibiting oxidative stress and activating the Nrf2 pathway (28). Finasteride reduces blood levels of DHT by inhibiting 5α-reductase activity, thereby reducing oxidative stress induced by DHT and preserving antioxidants (31). Medicinal plants employed for BPH treatment have been demonstrated to play a role in reducing plasma and prostate levels of DHT, which subsequently leads to a reduction in prostate weight and size (32). In accordance with prior research (32), an increase in PSA, DHT, prostate volume, and prostate index was observed in a rat BPH model. It is noteworthy that NSO treatment significantly mitigated these pathological phenomena, with doses of 400 and 800 mg/kg exhibiting effective results (33). Previous research by Hiipakka and colleagues demonstrated that green tea polyphenols were effective in reducing DHT production and prostate cell proliferation. The polyphenolic content of Nigella sativa may be linked to its beneficial effects, as it inhibits 5α-reductase. Abdel-Rahman et al. proposed that fatty acid-rich compounds, such as pumpkin seed, may impede prostate cell proliferation by reducing testosterone and DHT levels (34). Nanotechnology-based formulations derived from black cumin seed oil have garnered significant attention due to their distinctive characteristics, high biological efficacy, and stability (35-37). Given their high biological efficacy, nanoemulsions based on black cumin seed oil have recently attracted considerable interest. In comparison to conventional Q, its efficacy is primarily attributed to its high bioavailability, high surface activity, and low toxicity. Qu-NSO has been employed as a

nutritional supplement and in medical therapy due to its high stability. The development of benign prostatic hyperplasia (BPH) is significantly influenced by oxidative stress resulting from an imbalance between free radicals and antioxidants (38). The overproduction of reactive oxygen species during the inflammatory process results in oxidative stress, which in turn causes tissue damage and the development of oxidative-related diseases (39, 40). Patients with BPH frequently display elevated blood MDA levels, which serve as a marker of oxidative stress, along with reduced levels of antioxidant enzymes such as glutathione peroxidase and GSH (41). The objective of the study was to ascertain whether Qu-NSO could mitigate the oxidative damage induced by testosterone in this context. The study evaluated antioxidant enzyme levels and lipid peroxidation indices through the analysis of prostate tissue homogenates. The findings demonstrated that a 25 mg/kg Qu-NSO treatment diminished lipid peroxidation in prostate tissue by reducing MDA levels. The administration of Qu-NSO resulted in an elevation of SOD levels. The phytochemical analysis revealed that the rich phenolic and flavonoid content of Qu-NSO conferred potent antioxidant efficacy. These compounds, which are present in a variety of plants, have been demonstrated to exert a range of biological effects, including anti-inflammatory and antioxidant effects (42). Therefore, the antioxidant impact of Qu-NSO SNEDDS on BPH can be attributed to its phenolic and flavonoid content. These findings illustrate the potential of Qu-NSO to augment antioxidant status in testosterone-induced BPH rats, resulting in increased SOD and GPx levels. The elevated lipid peroxidation rates observed in the control group were effectively counterbalanced by significantly reduced rates in the black seed nanoemulsion with the Qu group, thereby reinforcing the protective antioxidant effects against BPH. The results indicate that the nano method may facilitate the beneficial effects of Qu on prostate hyperplasia markers while reducing the effective dose. The Qu-NSO combination proved more effective than finasteride in reducing prostatic weight and enhancing antioxidative properties, including increased antioxidant enzyme activity. This study demonstrated that the Qu and NSO in a Qu-NSO formula augmented the efficacy of Qu in managing BPH.

Acknowledgment
The Science and Research Branch of Islamic Azad University in Tehran, Iran, is gratefully acknowledged for its support of this work. The aforementioned research was conducted with the assistance of the Islamic Azad University, Tehran, Iran.

Authors' Contribution
All authors played a role in the conceptualization and design of the study. AJ was responsible for the preparation of materials and the acquisition of data, and was the primary author of the initial manuscript draft. NP was responsible for the study design, the conduct of the study, and the initial drafting of the manuscript. SH supervised the preparation of the pathology slides, while GA conducted the writing review, editing, and data analysis.

Ethics
The research project was approved by the Ethics Committee of the Science and Research Branch of the Islamic Azad University (code: IR.IAU.SRB.REC.1397.215). Moreover, in accordance with the tenets of the Helsinki Declaration, the research was conducted with due regard for the welfare of the animals involved.

Conflict of Interest
The authors certify that they have no conflicts of interest. The authors have no conflicts of interest to declare.

Funding
This research was not funded by a specific grant or other financial support.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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