



Quercetin nano phytosome: as a novel anti-leishmania and anti-malarial natural product

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Abstract

Leishmaniasis is a vector-borne disease that affects several populations worldwide with the clinical manifestations in skin, mucous membranes, and internal organs and there are not any effective and available vaccines and conventional treatments are highly toxic. Quercetin is a kind of flavonoid with different biological effects including free radical scavenging and anti-microbial activity and this study is aimed to assess the anti-leishmania and anti-malarial effects of quercetin loaded phytosome and quercetin alone. In this experimental study, the in vitro activity of above drugs were measured using microscopically examinations and for evaluation the anti-leishmanial efficacy, the size of lesions were measured. Moreover the cytotoxicity of the treatments was evaluated on WI38 and J774 cell lines. Our results indicated that quercetin loaded phytosome and quercetin alone have acceptable anti-parasitic activity mostly at concentration of 400 µg/ml on both *P. falciparum* and *L. major*. The results of cytotoxicity revealed that the mentioned drugs have no effects on human cell lines and also have no hemolytic activity. The drug of choice for the treatment of leishmaniasis, in addition to killing the parasite, should not have a toxic effect on human cells and our results indicated that quercetin can be a valuable candidate for treatment of different kinds of leishmaniasis.

Keywords Quercetin nano phytosome · Quercetin · Anti-leishmania · Anti-malaria · Natural product

Introduction

Leishmania and malaria are the most important vector-borne parasitic diseases that cause a large number of deaths in many parts of the world every year (Torres-Guerrero et al. 2017). Annually about 12 million people were infected by

Leishmaniasis mostly in developing countries (Steverding 2017). Leishmaniasis is transmitted via the bite of phlebotomine sand flies. Predominantly target reticuloendothelial cells and showed three more common manifestations including: Cutaneous Leishmaniasis (CL), visceral leishmaniasis (VL) and muco-cutaneous leishmaniasis (MCL) which CL

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is the most frequent form of disease that are self-limiting and may heal in 1–5 years (Pagheh et al. 2013). The clinical manifestations of the CL in including from a simple papule and early lesions to disseminated and mucosal lesions (Manna et al. 2009). Pentavalent antimonial are the golden standard treatment for different types of leishmaniasis but recent investigations reported some limitations such as high cost, toxicity, and resistance for use of these drugs (Raeisi et al. 2020).

Malaria is one of the most deadly and severe infectious diseases in the world caused by different species of *Plasmodium* SPP (Organization 2016). An investigation in 2016, reported 445,000 attributed globally deaths for malaria and around 216 million cases of malaria are in the world and children under 5 years and pregnant women in Africa are the most affected groups. *P. falciparum*, *P. malaria*, *P. vivax* and *P. ovale* are the most important causes of malaria in human (Taffese et al. 2018). The main problem in malaria is the development of drug resistance against this pathogen. In recent years, many drugs have been used to treat malaria. For example, drugs such as quinine, chloroquine, mefloquine, and in recent years, artemisinin have been the most popular drugs used, but Plasmodium parasite has become resistant to all of these drugs. Therefore, search for simple, safe, effective, cheap and accessible drugs for these infections is vitally required (Ashley and Phyto 2018; Alonso and Noor 2017).

Due to the occurrence of these problems for the use of chemical drugs, in recent years, medicinal plants and natural compounds have been considered as the main candidates to replace chemical drugs. Today, in many developed and developing countries, chemical drugs are widely used as antioxidant, anti-cancer, anti-fungal, anti-viral, anti-bacterial and anti-parasitic compounds (Jamshidi-Kia et al. 2018; Allen et al. 2020; Heydarian et al. 2019; Raeisi et al. 2020; Golami et al. 2016).

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-Hchromen-4-one) with a wide range of biological activities is Categorized as a polyphenolic flavonoid which is extracted from various vegetables and fruits with bright pharmacological actions, such as anti-microbe, antiviral, anti-inflammatory and anticancer (Ghadi et al. 2019; Sadeghi-Ghadi et al. 2020b). For example, anti-human T-lymphotropic virus 1, anti-Japanese encephalitis virus (JEV), anti-hepatitis C virus, anti-dengue virus type-2, anti-porcine epidemic diarrhea virus and anti-influenza-A virus effectiveness were reported for quercetin in different studies (Wu et al. 2016; Chiow et al. 2016). Also, it has been documented for its efficacy against the bacterial infections caused by *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Yersinia enterocolitica*, *S. aureus*, *Salmonella enterica*, *Escherichia coli*, fungal geniuses such as *Cryptococcus neoformans*, *Candida albicans*, *Aspergillus niger* and parasitic infections such as Babesiosis, theileriasis, trypanosomiasis, toxoplasmosis

and Leishmaniasis (da Silva et al. 2012; Fusetti et al. 2002; Schimmer et al. 1988; Vashisth et al. 2013). Studies of quercetin showed that quercetin is an exceptional natural compound and can be a source of hope for the treatment of important infections such as malaria and leishmaniasis.

Because of the absence of appropriate vaccines against leishmaniasis and malaria, anti-parasitic chemotherapy is the only weapon for controlling of these diseases. Moreover, production of new drugs is progressing very slowly; In this case, the best way to treat these patients is to use novel drug delivery techniques such as nanoparticle delivery systems, liposomes, emulsions, polymeric nanoparticles, noisome and phytosome to improving its efficiency. The aim of this study was to investigate the anti-malarial and anti-leishmaniasis effects of quercetin on *P. falciparum* and *L. major*.

Materials and methods

Materials

Quercetin (Alfa Aesar, Heysham, England) were purchased from Merck KGaA (Darmstadt, Germany). Hyaluronic sodium salt (HA-Na, Mw = 280 × 10³) was provided by Contipro (Dolní Dobrouč, Czech Republic).

Quercetin nano phytosome and particle size analysis

Thin layer hydration method with different concentrations of Quercetin, PC and cholesterol were used to prepare the Phytosomes. Quercetin and cholesterol were melted in methanol and dichloromethane, respectively in a round bottom flask and the solvent were evaporated using rotary evaporator (Heidolph, Germany), kept an overnight at 45 °C up to creating thin dry film in the bottom of the flask. The film was hydrated with distilled water in a rotary evaporator (BUCHI Rotavapor II) at 45 °C, sonicated (LABMAN LMUC-4) at 45 °C and homogenized with 20,000 rpm to decrease phytosomes size. The particle size analyzer (Wing SALD 2101, Japan) were used to measure the particle size and particle size distribution and stated by the volume median diameter (VMD) (Pourhajibagher et al. 2021).

Determination of encapsulation efficiency (EE)

An appropriate amount of dispersion was transferred in Millipore Amicon® Ultra filtration tube, centrifuged (Sigma-3 k-30, Germany) for 5 min at 5000 rpm and then the supernatant was collected and amount of free Quercetin was spectrophotometrically determined at 210 nm. The encapsulation efficiency has been determined according to the following equation (Cesarone et al. 2019):

$$EE(\%) = \frac{\text{Amount of the entrapped quercetin in the noisomes}}{\text{Total amounts of the quercetin}} \times 100$$

Parasites

L. major (MRHO/IR/75/ER) standard strain was collected from Leishmaniasis Research Center, School of Public Health, Tehran University of Medical Sciences, Iran and was cultured in RPMI-1640 medium (Sigma-Aldrich, Bornem, Belgium) supplemented with 10% heat-inactivated fetal bovine serum (Gibco from Thermo Fisher Scientific, Merelbeke, Belgium) and 1% penicillin/ streptomycin (Gibco 31,331, Breda, The Netherlands) at 37 °C, 5% CO₂. *P. falciparum* (Pf 3D7 strain) were obtained from Pasteur Institute of Iran and cultured in a modified medium contained of 16.20 g/L RPMI 1640 (Sigma, Munich, Germany), 11.11 mM glucose, 0.20% sodium bicarbonate (Sigma, Munich, Germany), 50 g/mL gentamicin (Gibco, Waltham, MA, USA), 45 g/mL hypoxanthine (Sigma, Munich, Germany), 25 mM HEPES, 0.50% Albumax I (Gibco, Waltham, MA, USA), and incubated at 37 C in an atmosphere of 5% O₂, 5% CO₂, and 90% N₂ (Raeisi et al. 2020; Elmi et al. 2021).

In vitro anti-leishmanial assay

The promastigotes density of *L. major* was 1×10^5 parasites/mL and all experiments were carried out in 96-well plates in triplicate. Quercetin loaded phytosome and quercetin (100 µL) in concentrations of 100, 200 and 400 µg/ml was added to culture medium and 100 µL of *L. major* promastigote in culture medium were added (total volume 200 µL). The viability of the promastigotes was measured using trypan blue as a vital staining. To calculate the IC₅₀, quercetin were used at concentrations ranging from 2.5 to 100 µg/ml and the IC₅₀ values were assessed from dose response growth inhibition curves by Microsoft excel software. Phosphate-buffered saline (PBS) and glucantime were used as negative and positive controls, respectively in each set of experiment (Esboei et al. 2018).

In vivo anti-leishmanial assay

Thirty BALB/c females at aged of 4–5 weeks and weighed 30–40 g were purchased from pasture Institute of Tehran (Tehran, Iran) and kept in standard conditions (light and temperature) and divided into 6 groups including 3 treatments group (100, 200 and 400 µg/ml), negative, positive and non-treated groups. *L. major* promastigotes (1×10^6) in stationary phase were intradermally injected in the tail base of each mouse. After 21 days, nodules and ulcers were appeared and the treatments were applied two times per

a day at the ulcer site and 150 µg/ml of treatments were used in each time using a cotton applicator for 4 weeks. The diameters of the lesions were measured using digital caliper and the mice were weighted by a digital scale weekly. All parts of the in vivo experiments were performed according to the ethically standards of animal and was approved by the Institutional Ethics Committee of Research of Islamic Azad University, Tonekabon branches (Esboei et al. 2018).

In vitro anti-plasmodial assay

100 µl of quercetin in concentrations of 100, 200, 400 µg/ml and Chloroquine (Sigma-Aldrich, New Delhi, India) and PBS as positive and negative groups, respectively were added to round-bottom 96-well. 100 µl of the parasites in culture medium were added. During incubation time, 100 L of SYBR green I lysis buffer (Tris (20 mM, pH 7.5), saponin (0.008%, w/v), Triton X-100 (0.08%, v/v) and EDTA (5 mM)) were added to the each well, lightly shaken and then incubated in dark at 37 C for 1 h. After incubation period, fluorescence intensity was then measured with a victor fluorescence multi-well plate reader () at 485 and 530 nm, respectively. The IC₅₀ values were calculated using the IC Estimator-version 1.2 software (<http://www.antimalarial-icestimator.net/MethodIntro.htm>) (Free Software Foundation, Boston, MA, USA) (Elmi et al. 2021).

Cytotoxicity assay

To assess the cytotoxicity effects of the mentioned drugs, Non-cancer (WI38) and cancer (J774) mammalian cell lines were cultured in DMEM medium supplemented with FCS 10% and CO₂ 5%. Diluted cells (100µL) at density of 1×10^4 cells/mL were added into each well of 96-well plates and incubated for 24 h at 37 °C. After incubation time, 100 µL of quercetin loaded phytosome and quercetin at concentrations of 400 µg/mL was added to each well and incubated for 72 h. Then, the medium was removed and 100 L of MTT was added to each well and incubated for 45 min. The MTT were discarded, 100 L of DMSO was added and the Absorbance was measured on a spectrophotometer at 570 nm. All experiments were done in triplicate (Zafari et al. 2017).

Hemolytic test

Five mL from human blood was collected and washed three times with Phosphate buffered saline (PBS). The quercetin loaded phytosome and quercetin alone at concentration of 400 µg/mL were prepared in microplate and triton X100 and PBS (Sigma-Aldrich, St. Louis, USA) were set as positive and negative controls, respectively. 100 µL Red blood cells (RBCs) suspension (2%) was added to each well and incubated for 2 h at 37 °C. after incubation time, The microplate

was centrifuged for 10 min at 1200 g and the absorbance of the supernatant was determined at 540 nm. The percentage of hemolysis for each sample was calculated by the following formula (Elmi et al. 2021):

$$\text{Hemolysis\%} = \frac{\text{Plasma hemoglobin concentration}}{\text{Total blood hemoglobin}}$$

Statistical analysis

Data were analyzed using SPSS v. 11.5 and the mean values less than 0.05 was accepted as statistical significant level. In this study mean values were analyzed by Two-way ANOVA and student's t-test.

Results

Particle size

The results indicated that the particle size was increased during 120 days and addition of cholesterol heighten the stability and endure at the proper size variety. The Particle size of nano phytosomes is a main factor the stability and bioavailability of phytoconstituent encapsulated systems. In current study different molar ratio of phosphatidylcholine to cholesterol were used and the results indicated that greater particles have later release as well as lowest stability and the characteristics of the phytosome, including particle sizes and encapsulation efficiency are different ($P=0.124$) (Table 1, Fig. 1).

Anti-leishmanial activity

The results of this study showed that quercetin loaded phytosome and quercetin was effective in all concentrations and

Table 1 Mean particle size and encapsulation efficiency of prepared Quercetin loaded phytosomes with different molar ratio

Molar ratio (QU: PC: CH)	Mean particle size (nm)				Encapsulation efficiency (EE) (%)	P value
	Day 1	Day 30	Day 60	Day 120		
1: 1: 0	86.53 ± 2.45	92.38 ± 1.96	98.18 ± 3.22	114.54 ± 2.33	98.67 ± 1.56	0.124
1: 2: 0	88.62 ± 1.29	174.43 ± 2.09	361.74 ± 3.61	443.76 ± 6.82	96.33 ± 0.87	
1: 2: 0.2	93.44 ± 3.51	93.76 ± 2.46	95.77 ± 2.65	96.66 ± 2.06	98.67 ± 0.89	

QU Quercetin, PC Phosphatidylcholine, CH cholesterol

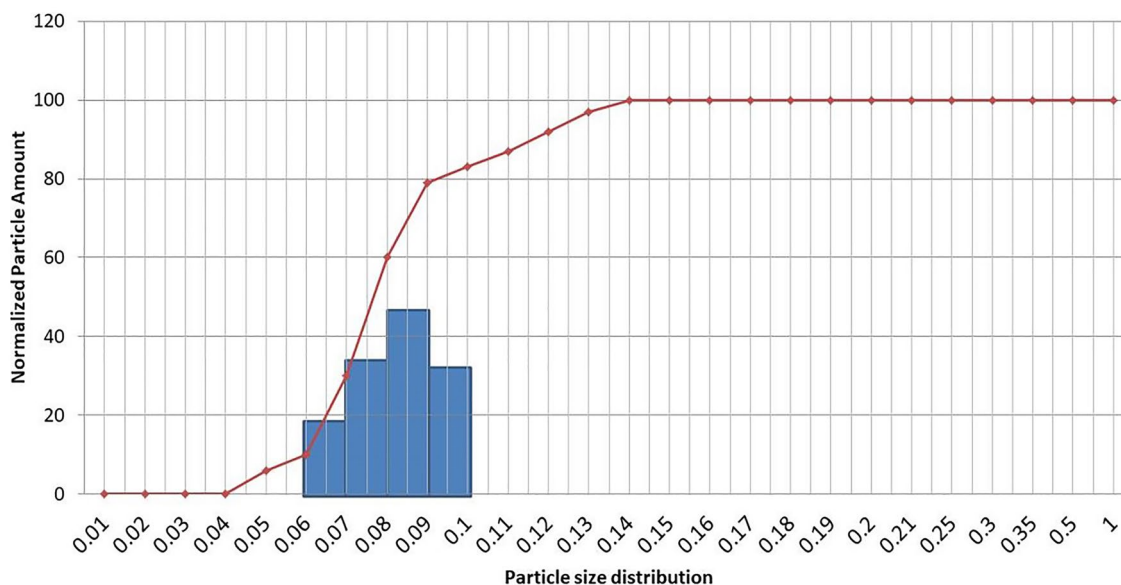


Fig. 1 Particle size distribution of nano phytosome

the concentration of 400 µg/ml at 72 h was the most effective dose and time. The differences between quercetin loaded phytosome at the concentration of 400 µg/ml have similar effectiveness to positive control but, the positive control showed better effectiveness than quercetin at the concentration of 400 µg/ml (Tables 2, and 3).

Anti-plasmodial activity

Anti-plasmodial effects of quercetin loaded phytosomes and quercetin alone was evaluated using microscopic and enzymatic methods (pfLDH). Our results indicated that the concentration of 400 µg/ml of both quercetin loaded

phytosomes and quercetin alone was the most effective dose that eliminate 92.31 and 78.87% of the parasites, respectively. Analysis of the results exhibited that there was no significant difference between microscopic and enzymatic methods results ($P > 0.05$). According to the Table 3, quercetin loaded phytosomes was more effective than quercetin alone in each time and concentrations which the differences were statically significant ($P < 0.05$) (Table 4).

Hemolytic activity

The Hemolytic Activity of the quercetin loaded phytosomes and quercetin alone were determined by MTT assay and

Table 2 The in vitro effectiveness of quercetin loaded phytosome and quercetin alone on *L. major* promastigote incubated for different periods. Data are expressed as the mean \pm SD (n = 3)

Drugs	Concentration	0 h	6 h	12 h	24 h	72 h	P value
Quercetin loaded phytosome	100 µg/ml	96.43	81.39 \pm 4.11	73.46 \pm 3.26	55.02 \pm 2.76	32.39 \pm 3.27	0.001
	200 µg/ml	97.68	77.38 \pm 5.33	68.28 \pm 4.72	44.29 \pm 3.48	19.44 \pm 2.84	
	400 µg/ml	96.34	61.47 \pm 5.84	34.77 \pm 4.37	3.32 \pm 2.31	00.00 \pm 00	
Quercetin	100 µg/ml	98.23	87.43 \pm 4.66	77.76 \pm 4.78	58.27 \pm 4.19	34.55 \pm 3.56	
	200 µg/ml	99.43	81.56 \pm 6.49	72.13 \pm 5.84	46.83 \pm 3.52	23.68 \pm 3.41	
	400 µg/ml	97.26	74.27 \pm 3.37	69.32 \pm 4.39	31.39 \pm 4.85	8.28 \pm 2.06	
NC		99.22	98.46 \pm 1.21	96.48 \pm 1.54	85.16 \pm 2.48	56.92 \pm 4.72	
PC		98.50	55.73 \pm 2.44	31.48 \pm 3.81	00.00 \pm 00	00.00 \pm 00	

NC Negative control, PC Positive control (Glucantime)

Table 3 The in vivo effectiveness of quercetin loaded phytosome and quercetin on *L. major* promastigote incubated for different periods. Data are expressed as the mean \pm SD (n = 5)

Drugs	Concentration	0 day	7 Days	14 Days	21 Days	28 Days	P value
Quercetin loaded phytosome	100 µg/ml	7.30 \pm 1.28	8.82 \pm 1.35	5.82 \pm 1.54	3.57 \pm 2.31	2.09 \pm 0.93	0.001
	200 µg/ml	6.14 \pm 2.41	5.73 \pm 2.56	3.48 \pm 2.88	3.15 \pm 1.49	0.33 \pm 1.22	
	400 µg/ml	8.05 \pm 2.43	5.37 \pm 2.66	2.71 \pm 1.21	0.94 \pm 0.32	0.00 \pm 0.00	
Quercetin	100 µg/ml	7.43 \pm 2.56	5.49 \pm 1.56	4.38 \pm 1.23	2.58 \pm 0.45	1.53 \pm 0.45	
	200 µg/ml	7.96 \pm 1.43	6.32 \pm 2.11	3.58 \pm 1.09	2.86 \pm 1.31	2.05 \pm 0.76	
	400 µg/ml	8.49 \pm 1.62	6.41 \pm 1.87	3.29 \pm 0.89	1.33 \pm 0.96	0.83 \pm 0.07	
NC		7.55 \pm 2.41	7.94 \pm 1.11	8.37 \pm 1.58	8.93 \pm 2.03	8.94 \pm 2.35	
PC		8.77 \pm 2.54	6.34 \pm 1.94	3.41 \pm 1.87	1.42 \pm 1.02	0.00 \pm 00	

NC Negative control, PC Positive control (Glucantime)

Table 4 The growth inhibition percentage of *Plasmodium falciparum* in different groups, in vitro

Drugs	Concentration	Growth inhibition percentage		P-value	IC50
		Mic test \pm SD	pfLDH \pm SD		
Quercetin loaded phytosome	100 µg/ml	59.51 \pm 1.43	61.06 \pm 2.43	0.131	84.27 µg/ml
	200 µg/ml	68.32 \pm 2.83	66.93 \pm 2.11	0.083	
	400 µg/ml	91.45 \pm 4.75	93.18 \pm 4.38	0.096	
Quercetin	100 µg/ml	43.78 \pm 2.43	42.49 \pm 3.21	0.321	179.38 µg/ml
	200 µg/ml	52.49 \pm 3.76	51.05 \pm 2.41	0.098	
	400 µg/ml	78.32 \pm 2.77	79.43 \pm 2.74	1.432	
NC		1.48 \pm 0.07	1.87 \pm 0.84	0.152	–
PC		97.55 \pm 2.48	96.37 \pm 3.29	0.473	–

NC Negative control, PC Positive Control (Glucantime)

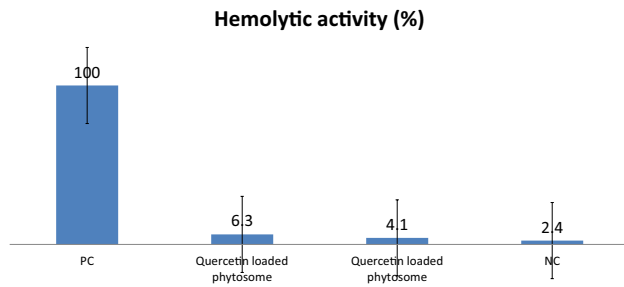


Fig. 2 The Hemolytic effectiveness of the quercetin loaded phytosomes and quercetin alone in comparison to the triton 100X and PBS as positive and negative control groups, respectively

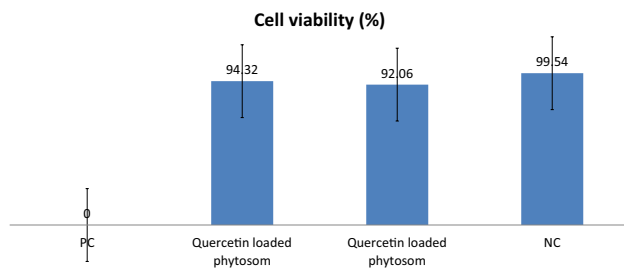


Fig. 3 The cytotoxicity effectiveness of the quercetin loaded phytosomes and quercetin alone on WI38 and J774 cell lines

the results revealed that quercetin loaded phytosomes and quercetin alone at the highest concentration (400 µg/ml) have 6.3 and 4.1% hemolytic effects. Whereas triton 100X as positive control showed 100% hemolytic effects with significant differences to the test groups ($P < 0.001$) (Fig. 2).

Cell viability assay

The results revealed that the cell viability of non-cancer (WI38) and cancer (J774) mammalian cell lines after exposure to the concentrations of 400 µg/mL of quercetin loaded phytosomes and quercetin alone were 94.32 and 92.06%, respectively (Fig. 3).

Discussion

The speedily increasing expansion of antimalarial and anti-leishmanial drug resistance during the earlier decades caused problematic treat for parasitic infections, so it's give emphasis to search for new anti-parasitic agents (Esboei et al. 2018). In current study, Quercetin is used to evaluate its anti-parasitic and cytotoxicity effects. Quercetin is kind of flavonoids and poor lipid solubility, low absorption, low bio-availability and large size are the main limitations of these compounds, so in current investigation we loaded it into

the phytosome and developed a new formulation (Sadeghi-Ghadi et al. 2020a). The results of many published studies revealed that drugs in nano-size are significantly better in crossing penetrability cell walls, in comparison with normal forms. Suryawanshi et al. (2011) stated that Phytosome as like as liposome is a novel botanical formulation to produce lipophilic molecular complex that can significantly increases the bioavailability and absorption of phytoconstituent particularly polyphenolics (Suryawanshi 2011).

In this study, quercetin alone and nanoquercetin loaded phytosomes were compared to evaluate their antiparasitic effects, and before these experiments, the physical properties of the nano drug were evaluated. At first, changes in nano-particle size were investigated and the results showed that this compound did not significantly changed in size during 120 days. Our results indicated that quercetin loaded phytosome have both anti-leishmanial and anti-malarial effects in vitro and in vivo by inhibiting growth and against the leishmania and plasmodium parasite with no cytotoxicity on human normal cell line. In spite of several studies about Quercetin's antifungal and anti-bacterial effects, no study has been described about the anti-plasmodium and anti-leishmanial effect on *P. falciparum* and *L. major* parasites.

Quercetin loaded phytosome were successfully prepared using thin film hydration method with 15 nm homogenous size after loading of quercetin measured by DLS method and without aggregation. Phytosomes must be stable during the storage period and remain at the appropriate size range before reaching their targeted tissues when used as a drug delivery system. Physical stability of optimum formulation, has been studied for seven days and results indicated to instability due to the size increasing.

Quercetin showed anti-oxidant activity by scavenging oxygen radicals (Chow et al. 2005), protecting lipids against peroxidation (Terao et al. 1994), have anti-inflammatory properties by inhibition the production of TNF- α 14 and IL-8 (Lesjak et al. 2018). Some researchers have investigated the antibacterial effects of quercetin. Li and Xu (2008) indicated that quercetin were had antibacterial effects against *A. actinomycetemcomitans*, *A. viscosus*, *P. gingivalis*, *F. nucleatum*, and *A. naeslundii* with the minimum inhibitory concentrations of 0.625, 1.25, 1.25, 0.625 and 2.5 mg/mL, respectively (Li and Xu 2008). In a similar study conducted by Jaisinghani in 2017 quercetin showed *P. aeruginosa*, *P. vulgaris*, *E. coli* and *Shigella flexneri* were inhibited at the concentrations of 20, 300, 400 and 500 mg/mL, respectively (Jaisinghani 2017). By comparing these results, it could concluded that bacteria geniuses are more vulnerable than parasites.

Maramaldi et al. (2016) indicated that quercetin phospholipids 1% has a significant soothing effect against skin inflammation induced by various insults, comprising histamine prick test, UV radiation, skin barrier interruption

induced by contact with sodium lauryl sulfate or glycolic acid solutions in external use. Moreover it's suggested that quercetin has skin protective effects and can cause reduction in skin irritation by anti-allergy action and inhibition of cytokine production (Maramaldi et al. 2016). 3-chymotrypsin-like protease (3CLpro), papain-like protease (PLpro), RNA-dependent RNA polymerase, and spike (S) proteins are the main viral proteins for SARS-CoV-2 that are the candidate for the treatment of COVID-19 disease (Derosa et al. 2021). Pierro et al. (2020) in a molecular docking study revealed that quercetin can inhibit 3CLpro, PLpro and S proteins (Di Pierro et al. 2020).

Three concentrations of quercetin loaded phytosome were applied in current study against *L. major* and *P. falsiparum* and their activity as well as their cytotoxicity is demonstrated in Tables 2 and 3. The concentration of 400 µg/ml showed surprising effectiveness on *L. major* in vitro and in vivo experiments. This concentration killed 100% of the promastigotes of the *L. major* in vitro and completely treated the leishmaniasis lesion in vivo. Quercetin alone at concentration of 400 µg/ml killed 74.27 ± 3.37 , 69.32 ± 4.39 , 31.39 ± 4.85 and $8.28 \pm 2.06\%$ of the *L. major* promastigote after 6, 12, 24 and 72 h, respectively. According to our expectation quercetin loaded phytosome showed better effectiveness in comparison to the quercetin alone in all concentrations and the difference was statically significant ($P=0.025$).

In this study, in addition to the anti-leishmaniasis effect, the anti-malarial effect of the drugs was also investigated against *P. falciparum*. Our results indicated that both quercetin loaded phytosome and quercetin alone have anti-plasmodium activity in all concentrations with the IC50 of 84.27 and 179.38 µg/ml, respectively of course, the concentrations of 400 µg/ml was the most effective dose. In testing of anti-plasmodium effectiveness, both quercetin loaded phytosome and quercetin alone showed similar activity on ring stage of *P. falciparum* ($P>0.05$). By comparing the results, it showed that the *P. falciparum* parasite was more sensitive than the *L. major* parasite and the antiparasitic effect of the drugs used in this study had a better antimalarial effect. The purpose of using two drugs simultaneously was to investigate the mutual effect of two drugs and the results showed that the simultaneous use of two drugs had a synergistic effect.

The cytotoxicity results proved the non-toxicity of quercetin loaded phytosome and quercetin alone at high concentration (400 µg/mL). In a study conducted by Maiyo et al. (2016) showed quercetin have much lower cytotoxicity against the HEK293 cell line with IC50 values of 186 µg mL(-1)(C Maiyo, Moodley and Singh 2016). In a similar study by Kusaczuk et al. (2022) our results was approved and showed that quercetin was safe in confronting with A172 cells (Kusaczuk et al. 2022).

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Author contributions FJJ, HH and BRE: Designed and performed experiments, analyzed data and co-wrote the paper. HH, VA, HV and BRE: Performed experiments. MK: Performed bioinformatics analyses. BRE, HH and ES: provided essential mouse strains HV and FJJ: Supervised the research. VA, HV, MN and BRE: Designed experiments and co-wrote the paper.

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Data availability All extracted data from the current study is mentioned in this manuscript and there is not any supplementary data.

Declarations

Conflict of interest There is not any conflict of interests.

Ethical approval This study is ethically approved by Ethic committee of Islamic Azad University, Tonekabon branch with the number of: IAU.TON.1400-031.

Consent for publication All authors are in agreement to publish this manuscript in Pharmaceutical Medicine journal.

References

- Allen WE, Altae-Tran H, Briggs J, Jin X, McGee G, Shi A, Raghavan R, Kamariza M, Nova N, Pereta A (2020) Population-scale longitudinal mapping of COVID-19 symptoms, behaviour and testing. *Nat Hum Behav* 4:972–982
- Alonso P, Noor AM (2017) The global fight against malaria is at crossroads. *The Lancet* 390:2532–2534
- Ashley EA, Phyo AP (2018) Drugs in development for malaria. *Drugs* 78:861–879
- Cesarone MR, Belcaro G, Hu S, Dugall M, Hosoi M, Ledda A, Feragalli B, Maione C, Cotellese R (2019) Supplementary prevention and management of asthma with quercetin phytosome: a pilot registry. *Minerva Med* 110:524–529
- Chiew K, Phoon M, Putti T, Tan BK, Chow VT (2016) Evaluation of antiviral activities of *Houttuynia cordata* Thunb. extract, quercetin, quercitrin and cinanserin on murine coronavirus and dengue virus infection. *Asian Pac J Trop Med* 9:1–7
- Chow J-M, Shen S-C, Huan SK, Lin H-Y, Chen Y-C (2005) Quercetin, but not rutin and quercitrin, prevention of H₂O₂-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. *Biochem Pharmacol* 69:1839–1851
- da Silva ER, do Carmo Maquiaveli C, Magalhães PP (2012) The leishmanicidal flavonols quercetin and quercitrin target *Leishmania* (*Leishmania*) *amazonensis* arginase. *Exp Parasitol* 130:183–188
- Derosa G, Maffioli P, D'Angelo A, Di Pierro F (2021) A role for quercetin in coronavirus disease 2019 (COVID-19). *Phytother Res* 35:1230–1236
- Di Pierro F, Khan A, Bertuccioli A, Maffioli P, Derosa G, Khan S, Khan BA, Nigar R, Ujjan I, Devraian BR (2020) Quercetin phytosome® as a potential drug for Covid-19. *Minerva Gastroenterol e Dietol* 67(2):190–195
- Elmi T, Esboei BR, Sadeghi F, Zamani Z, Didehdar M, Fakhar M, Chabra A, Hajjalani F, Namazi MJ, Tabatabaie F (2021) In vitro antiprotozoal effects of nano-chitosan on plasmodium falciparum,

- giardia lamblia and trichomonas vaginalis. *Acta Parasitol* 66:39–52
- Esboei BR, Mohebbali M, Mousavi P, Fakhar M, Akhoundi B (2018) Potent antileishmanial activity of chitosan against Iranian strain of *Leishmania major* (MRHO/IR/75/ER): in vitro and in vivo assay. *J Vector Borne Dis* 55:111
- Fusetti F, Schröter KH, Steiner RA, van Noort PI, Pijning T, Rozeboom HJ, Kalk KH, Egmond MR, Dijkstra BW (2002) Crystal structure of the copper-containing quercetin 2, 3-dioxygenase from *Aspergillus japonicus*. *Structure* 10:259–268
- Ghadi ZS, Dinarvand R, Asemi N, Amiri FT, Ebrahimnejad P (2019) Preparation, characterization and in vivo evaluation of novel hyaluronan containing niosomes tailored by Box-Behnken design to co-encapsulate curcumin and quercetin. *Eur J Pharm Sci* 130:234–246
- Golami S, Rahimi-Esboei B, Mousavi P, Marhaba Z, Youssefi MR, Rahimi MT (2016) Survey on efficacy of chloroformic extract of *Artemisia annua* against *Giardia lamblia* trophozoite and cyst in vitro. *J Parasit Dis* 40:88–92
- Heydarian P, Nateghpour M, Mazhari N, Motevalli Haghi A, Farivar L, Souri E, Javadi Mamaghani A, Rahimi Esboei B (2019) Evaluation of effectiveness of ethanolic extract of curcuma longa, discretely and in combination with chloroquine against chloroquine-sensitive strain of plasmodium berghei. *Herb Med J* 3:133–138
- Jaisinghani RN (2017) Antibacterial properties of quercetin. *Microbiol Res* 8:13–14
- Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H (2018) Medicinal plants: Past history and future perspective. *J Herbmed Pharmacol* 7:1–7
- Kusaczuk M, Krętownski R, Naumowicz M, Stypułkowska A, Cechowska-Pasko M (2022) A preliminary study of the effect of quercetin on cytotoxicity, apoptosis, and stress responses in glioblastoma cell lines. *Int J Mol Sci* 23:1345
- Lesjak M, Beara I, Simin N, Pintač D, Majkić T, Bekvalac K, Orčić D, Mimica-Dukić N (2018) Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *J Funct Foods* 40:68–75
- Li M, Xu Z (2008) Quercetin in a lotus leaves extract may be responsible for antibacterial activity. *Arch Pharmacol Res* 31:640–644
- Maiyo C, Moodley FR, Singh M (2016) Cytotoxicity antioxidant and apoptosis studies of quercetin-3-O glucoside and 4-(β-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)-benzyl isothiocyanate from *Moringa oleifera*. *Anti Cancer Agents Med Chem (formerly Current Medicinal Chemistry-Anti-Cancer Agents)* 16:648–656
- Manna L, Reale S, Vitale F, Gravino AE (2009) Evidence for a relationship between *Leishmania* load and clinical manifestations. *Res Vet Sci* 87:76–78
- Maramaldi G, Togni S, Pagin I, Giacomelli L, Cattaneo R, Eggenhöfner R, Burastero SE (2016) Soothing and anti-itch effect of quercetin phytosome in human subjects: a single-blind study. *Clin Cosmet Investig Dermatol* 9:55
- Pagheh AS, Fakhar M, Mesgarian F, Rahimi-Esboei B, Badiie F (2013) Incidence trend of rural cutaneous leishmaniasis in Gonbad-e-Qabus city, (Golestan, Iran) during 2009–2012. *J Mazandaran Univ Med Sci* 23:27–33
- Pourhajibagher M, Rahimi-Esboei B, Ahmadi H, Bahador A (2021) The anti-biofilm capability of nano-emodin-mediated sonodynamic therapy on multi-species biofilms produced by burn wound bacterial strains. *Photodiagn Photodyn Ther* 34:102288
- Raeisi M, Mirkarimi K, Jannat B, Rahimi Esboei B, Pagheh AS, Mehrbakhsh Z, Ghaffarifar F, Jorjani O, Foroutan M (2020) In vitro effect of some medicinal plants on leishmania major strain MRHO/IR/75/ER. *Med Lab J* 14:46–52
- Sadeghi-Ghadi Z, Ebrahimnejad P, Talebpour Amiri F, Nokhodchi A (2020a) Improved oral delivery of quercetin with hyaluronic acid containing niosomes as a promising formulation. *J Drug Target* 29:1–10
- Sadeghi-Ghadi Z, Vaezi A, Ahangarkani F, Ilkit M, Ebrahimnejad P, Badali H (2020b) Potent in vitro activity of curcumin and quercetin co-encapsulated in nanovesicles without hyaluronan against *Aspergillus* and *Candida* isolates. *J Mycol Med* 30:101014
- Schimmer O, Häfele F, Krüger A (1988) The mutagenic potencies of plant extracts containing quercetin in *Salmonella typhimurium* TA98 and TA100. *Mutat Res Genet Toxicol* 206:201–208
- Steverding D (2017) The history of leishmaniasis. *Parasit Vectors* 10:1–10
- Suryawanshi JS (2011) Phytosome: an emerging trend in herbal drug treatment. *J Med Genet Genomics* 3:109–114
- Taffese HS, Hemming-Schroeder E, Koepfli C, Tesfaye G, Lee M-C, Kazura J, Yan G-Y, Zhou G-F (2018) Malaria epidemiology and interventions in Ethiopia from 2001 to 2016. *Infect Dis Poverty* 7:1–9
- Terao J, Piskula M, Yao Q (1994) Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. *Arch Biochem Biophys* 308:278–284
- Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R (2017) Leishmaniasis: a review. *F1000Research* 6:750
- Vashisth P, Nikhil K, Pemmaraju SC, Pruthi PA, Mallick V, Singh H, Patel A, Mishra NC, Singh RP, Pruthi V (2013) Antibiofilm activity of quercetin-encapsulated cyto-compatible nanofibers against *Candida albicans*. *J Bioact Compat Polym* 28:652–665
- WH Organization (2016) World malaria report 2015. World Health Organization
- Wu W, Li R, Li X, He J, Jiang S, Liu S, Yang J (2016) Quercetin as an antiviral agent inhibits influenza A virus (IAV) entry. *Viruses* 8:6
- Zafari F, Sadeghi M, Moghanloo E, Bakhtiyari M (2017) Assessment of cd93 stem cell growth and survival on three-dimensional biodegradable pcl-gelatin scaffold. *J Mazandaran Univ Med Sci* 26:211–218

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