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Enhanced Bioavailability and Pharmacokinetics of a Natural Self-Emulsifying Reversible Hybrid-Hydrogel System of Quercetin: A Randomized Double-Blinded Comparative Crossover Study

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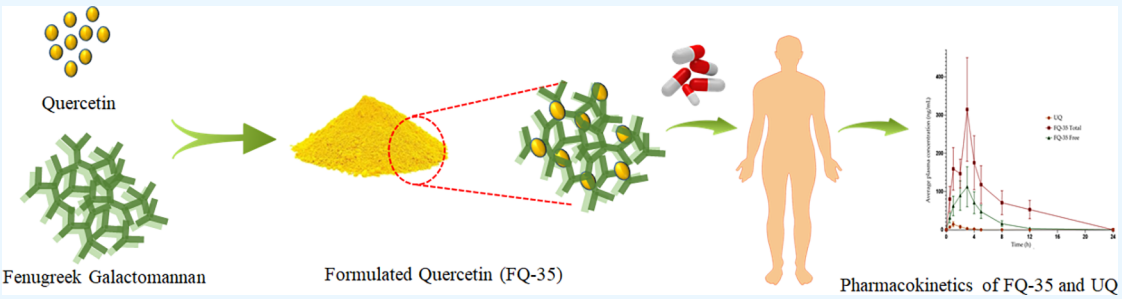

ABSTRACT: Despite the vast array of health beneficial pharmacological effects, the bioavailability of the dietary flavonoid quercetin was found to be poor due to insolubility, incompatibility, and rapid biotransformation. Herein, we investigated the solubility, morphology, particle size, stability, *in vitro* release, and human pharmacokinetics of a hybrid-hydrogel formulation of quercetin (FQ-35) using fenugreek galactomannans as the hydrogel scaffold. Physicochemical characterization revealed that the crystalline quercetin was well encapsulated in the hydrogel matrix to form translucent microgel particles of FQ-35 with enhanced solubility (96-fold). The mean particle size was found to be 183.6 ± 42.7 nm with a zeta potential of 35.1 ± 3.8 mV. Pharmacokinetic investigation on healthy volunteers (N = 16) employing tandem mass spectrometric (ultra-performance liquid chromatography-electrospray tandem mass spectrometry) measurements of the concentration of free (unconjugated) and conjugated quercetin metabolites revealed an 18.6-fold improvement in free (unconjugated) quercetin bioavailability and 62-fold improvement in total quercetin (sum of free and conjugated) bioavailability, compared to the unformulated quercetin extracted from Sophora japonica. In summary, the natural self-emulsifying reversible hybrid-hydrogel delivery system was found to offer significant solubility, stability, and bioavailability of quercetin upon single-dose oral administration.

1. INTRODUCTION

The health beneficial pharmacological activities and extreme safety profile of quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4*H*-chromen-4-one) have widely been established, and it is one of the most favored dietary flavonoids.[1,2] It is mainly used as a dietary antioxidant or as an anti-allergic/antiviral agent in many drugs and nutraceuticals approved by regulatory agencies like the U.S. Food and Drug Administration (USFDA) and European Food Safety Authority (EFSA).[2,3] Quercetin has been shown to stimulate the immune system and inhibit histamine release, pro-inflammatory cytokines, leukotriene synthesis, and inflammatory mediator enzymes like lipoxygenase, eosinophil, and peroxidase.[4] It was shown to disturb the viral life cycle steps including the synthesis, binding, and assembly of mRNA and negative strands.[5] *In silico* evaluation of natural product libraries has identified quercetin as one among the top five potent compounds to bind to the spike protein receptor of novel SARS-CoV-2 virus.[6,7] It has also exhibited even greater affinity than hydroxychloroquine toward the COVID-19 protease active site.[8,9] Recently, it was reported that quercetin has a synergistic antiviral effect when co-administrated with vitamin C and reduces severity conditions associated with earlier stages of COVID-19 infection.[2] In addition, co-administration of quercetin with antiviral drugs was found to offer significant reduction in the hospitalization period of COVID-19 patients.[10] Quercetin was also demonstrated for cardioprotective, neuroprotective, gastroprotective, anticarcinogenic, antimicrobial, anti-atherosclerotic, anti-inflammatory, immunomodulatory, antihypertensive, anti-obesity, antihyperglycae-

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mic, and bone-protective properties.[11] Quercetin has also protected neurons from oxidative damage and shows inhibition of β-amyloid protein fibril formation.[12] The antiproliferative effect of quercetin to induce cell cycle arrest and inhibit migration and growth has also been illustrated through various pathways.[13,14]

Owing to the pharmacological effects, safety and health benefits, there is a great interest for quercetin in nutraceuticals and functional foods for both prophylaxis and treatment.[15,16] In 2021, the market for quercetin was estimated at about $260 million, with an average growth of 6.53% to reach $400 million by 2025.[17] However, the functional benefits of quercetin have been limited due to its poor solubility (1 μg/mL), instability under gastrointestinal conditions, low oral bioavailability (<2%), and short biological half-life.[13,18] Quercetin undergoes extensive first-pass metabolism in the small intestine, colon, liver, and kidney and is converted mainly into sulfated and glucuronidated forms (conjugated metabolites).[1,18]

Nanodelivery systems have been shown to be of promise for therapeutic molecules from being enzymatically metabolized, henceforth, increasing its stability and systemic circulation time.[19] Different nanoencapsulation technologies including liposomes, emulsions, solid lipid nanoparticles, and polymeric micelles have been reported for quercetin.[20−23] Such encapsulation techniques will enhance the stability, solubility, dispersibility, and hence the bioavailability in animal studies. However, these formulations possess many limitations for food and nutrition applications, mainly due to the regulatory issues contributed by the extensive usage of synthetic excipients and non-food grade status. Poor thermodynamic stability, shorter storage shelf life, low loading levels, difficulty in scaling up, and high cost are some other drawbacks often associated with such nanoformulations. Recently, hydrogels are emerging as potential candidates for oral delivery.[24] Hybrid-hydrogels incorporated with nano/microstructures like liposome/micelles are intelligent drug delivery systems to enhance not only the therapeutic efficacy but also for the sustained release of the drug molecules.[25,26] Recently, we reported a hybrid-hydrogel system for vitamin C and resveratrol with improved stability, solubility, and hence enhanced human bioavailability using fenugreek (*Trigonella foenum-graecum*) galactomannan (FG) as the hydrogel scaffold.[27,28] The formulation was characterized as a food-grade and commercially viable "natural self-emulsifying reversible hybrid-hydrogel" (N'SERH) delivery system. In the present study, we aimed at the investigation of the solubility, particle size analysis, *in vitro* release profile, stability, and human pharmacokinetics of a N'SERH formulation of quercetin (FQ-35).

2. MATERIALS AND METHODS

2.1. Preparations of Hybrid-Hydrogel of Quercetin. The hybrid-hydrogel form of quercetin (FQ-35) was prepared according to a previously reported method of the N'SERH delivery system with slight modifications.[28] A gel-phase dispersion technique followed by vacuum drying was used for the preparation. First, micelles were prepared by heating about 40 g of quercetin with 5 g of sunflower oil and 20 g of lecithin to form a homogeneous mass. It was then suspended in ethanol-water (25/75, v/v) solution and further homogenized to form a clear solution. Ethanol was then evaporated under vacuum to form quercetin micelles. The micelles were further encapsulated into the fenugreek galactomannan hydrogel scaffold by homogenization and dehydration to obtain FQ-35 as a powder. Fenugreek galactomannan hydrogel showed enhanced surfactant properties when coming in contact with lecithin, and thus, FQ-35 exhibited instant water dispersibility and stability to act as a self-emulsifying system.

The encapsulation efficiency of the micellar quercetin within the hydrogel scaffold was further estimated using the ultracentrifugation technique.[29] Briefly, the sample was centrifuged at 9000*g* for 30 min and the content of quercetin in the supernatant was analyzed by a validated HPLC method.[30] The encapsulation efficiency was calculated using the equation

$$\text{encapsulation efficiency} = \frac{\text{total amount of quercetin in the formulation} - \text{free quercetin}}{\text{total amount of quercetin in the formulation}} \times 100\%$$

2.2. General. Unformulated quercetin and its proprietary hybrid-hydrogel formulation (FQ-35) were produced in the GMP (good manufacturing practice)-certified manufacturing plant of Akay Natural Ingredients, Cochin, India and were obtained as a granular powder with the certificate of analysis indicating the food-grade status and suitability for human consumption. The quercetin content was analyzed using high-performance liquid chromatography (HPLC) with a photodiode array detector and reverse phase C18 Phenomenex column (250 × 4.6 mm, 3 μm (Nexera X2, Shiamdzu, Kyoto, Japan)). Analytical standards of quercetin (CAS no. 117-39-5) and salbutamol (CAS no. 18559-94-9) were obtained from Sigma-Aldrich. LC−MS grade solvents were used for the analysis. Hydrodynamic size distribution and the zeta potential were measured using the dynamic light scattering (DLS) technique (Horiba SZ-100 particle size analyzer, Horiba, Kyoto, Japan). The morphological properties were characterized by the scanning electron microscopic (SEM) technique (Jeol 6390 LA SEM, JEOL Ltd., Tokyo, Japan) and transmission electron microscopy (JEM-2100 instrument, JEOL Ltd., Tokyo, Japan).

2.3. *In Vitro* Release Kinetics. *In vitro* release studies of quercetin from FQ-35 were performed in USP dissolution apparatus (Electro lab, Mumbai, India), and the release profile was studied at different pH (pH 6.5 and 3), maintaining the temperature at 37 ± 0.5 °C as reported earlier.[28] The released quercetin content in the sample was estimated by HPLC. Each experiment was performed in triplicate, and the average values were calculated.

2.4. Pharmacokinetics Study Design and Subjects. A randomized, double-blinded, placebo-controlled, two-way, two-sequence single-dose crossover design was adopted to determine the pharmacokinetics of FQ-35 in comparison with the unformulated standard quercetin (UQ). Identical hard shell gelatin capsules containing 600 mg of either UQ or FQ-35 were ingested, and the plasma concentration of the quercetin content was estimated at regular post-administration time points, 0, 0.5, 1,2,3, 4, 5, 8, 12, and 24 h.

The study was approved by a registered ethical committee and was performed according to the clinical research guidelines of the Government of India (CTRI/2021/06/034081, dated 08/06/2021) and in compliance with the declaration of Helsinki. Nineteen healthy volunteers (age group 21−56 years) who showed interest to take part in the study were screened based on a standard clinical assessment comprising a diagnostic interview and medical history analysis, and 16 were

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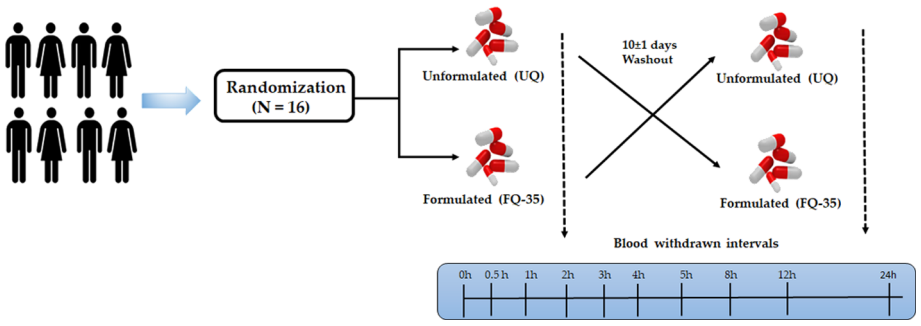

Figure 1. Diagrammatic representation of the study protocol.

enrolled for the study. Subjects having symptoms of viral infection and known hypersensitivity to the investigational products were also excluded. All the volunteers were advised to avoid quercetin containing supplements and food items such as onion, berries, apple, tea, and nuts prior to 2 days of the study. Lactating and pregnant women were omitted from the study.

Figure 1 shows the schematic illustration of the study design. The selected volunteers were randomly assigned into two different groups and provided with either the FQ-35 or UQ. The subjects reported to the study site by 7−8 a.m. in a fasting stage. Almost 5 mL of the blood sample was collected into heparinized tubes before dosing and designated post-administration time intervals employing an indwelling venous cannula. The collected blood samples were centrifuged (11,950*g*) for 10 min at 4 °C and stored at −20 °C for analysis.

2.5. UPLC-ESI-MS/MS Analyses of the Quercetin Content in Plasma. The quercetin content in blood plasma was identified and quantified with ultrahigh pressure liquid chromatography coupled with an electrospray ionization technology (UPLC-ESI-MS/MS, Quad-6460, Agilent technologies, Singapore) using multiple reaction monitoring (MRM). Separation of quercetin was done with an Agilent RRHD C18, LC column (100 × 3 mm, 1.8 μm) and kept at 35 ± 1 °C. The binary mobile phases (A and B) used for the chromatographic separation consisted of (A) acidified water (0.2% formic acid) and (B) acetonitrile containing 0.2% formic acid at a flow rate of 0.2 mL/min. Data was acquired using Mass hunter software version B.08.02. The linearity and the range of the extraction efficiency were evaluated by spiking 20 ng/mL quercetin in plasma along with salbutamol (internal standard) (10 ng/mL) followed by LC−MS/MS analysis. The precision and accuracy of the method were within the tolerable limits (15%) according to the ICH guidelines.

Extraction of quercetin from plasma was performed using ethyl acetate as described previously.[30] Briefly, 2 × 1 mL ethyl acetate was employed to extract 1 mL of plasma by high-speed mixing for 1 min and centrifuged at 9000*g* for 10 min at 4 °C and the top layer was collected. The procedure was performed in triplicate and filtered using a PVDF syringe filter (0.45 μm); 6 μL was injected. The mean plasma concentration−time curve was plotted, and the main pharmacokinetic parameters C_{max} (maximum plasma concentration), t_{max} (time to achieve C_{max}), AUC (area under the plasma concentration vs time curve), and $t_{1/2}$ (elimination half-life) were further calculated by a noncompartmental model.

The free quercetin content in plasma was directly estimated without enzymatic hydrolysis using glucuronidase. However, the measurement with enzymatic hydrolysis provided the total quercetin content (sum of glucuronidated/sulfated conjugates and free form) in the plasma since the enzymes convert the glucuronides and sulfates to the free form. Thus, the difference between the two measurements can lead to the quantification of conjugated forms. β-Glucuronidase (CAS no. G7017; Sigma-Aldrich) was employed for the analysis.

2.6. Statistical Analysis. The data were analyzed using SPSS (Statistical Package for the Social Science) software (version 27) and presented as mean ± standard deviation. Significant difference of plasma pharmacokinetic parameters was determined using analysis of variance (ANOVA) followed by Dunnett's test to evaluate the differences between the groups. A "*P*" value less than 0.05 was considered statistically significant. All data were processed using Graph Pad Prism Version 5.0.

3. RESULTS

Quercetin extracted from the flower buds of Sophora japonica by an ethanol-water extraction and crystallization process (unformulated quercetin, UQ) and its formulation (FQ-35) were used for the pharmacokinetic study. HPLC analysis showed a 98.04% quercetin content in UQ and 35.5% in FQ-35. A previously reported method of the natural self-emulsifying reversible hybrid-hydrogel (N'SERH) delivery system was employed for the formulation of FQ-35 by incorporating quercetin micelles into a hydrogel scaffold followed by dehydration into powder form such that it can instantly disperse in water or gastrointestinal fluid to form a soft hydrogel solution and hence to release the encapsulated quercetin molecules. The particle size in solution when measured by the dynamic light scattering technique indicated 183.6 ± 42.7 nm. Upon dehydration under vacuum, the gel solution can be converted back to powder. Thus, FQ-35 is a self-emulsifying reversible hybrid-hydrogel system prepared with naturally occurring fenugreek galactomannan as the hydrogel scaffold.

The physical and chemical parameters of UQ and FQ-35 are illustrated in Table 1. When the solubility was analyzed at pH 6.8 employing the method of Riva et al.,[30] the solubility of quercetin in FQ-35 was found to be increased by 96-fold (2.8912 mg/mL), compared to UQ (0.0302 mg/mL) [Figure 2(i)]. The encapsulation efficiency of quercetin micelles in fenugreek galactomannan hydrogel was determined to be 96.76 ± 1.73%. The hydrodynamic size of the FQ-35 solution prepared by ultrasonication showed particles of about 183.6 ± 42.7 nm [Figure 2(ii)] with a zeta potential of 35.1 ± 3.8 mV. The TEM image at different magnifications further confirmed the spherical morphology of the particles with size <200 nm [Figure 2(iii)]. The *in vitro* release profile of quercetin from FQ-35 is presented in Figure 3, which showed the sustained

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Table 1. Physicochemical Parameters of the Test Substances Used in the Present Study

parameters	unformulated quercetin (UQ)	formulated quercetin (FQ-35)
color and appearance	light yellow color and free flowing powder	light yellow color and free flowing granular powder
tapped density	0.29 g/mL	0.63 g/mL
total quercetin content	98.4%	36.3%
moisture	0.6%	2.1%
heavy metals		
lead	0.098 ppm	0.051 ppm
mercury	ND*	ND
cadmium	ND	ND
arsenic	0.10 ppm	0.06 ppm
pesticides	ND-USP$	ND-USP$
mycotoxins		
B1 + B2 and G1+ G2	<4.0 ppb	<4.0 ppb
aflatoxin B1	<2.0 ppb	<2.0 ppb
ochratoxin	<15.0 ppb	<15.0 ppb
microbiology		
total plate count[a]	500 cfu/g	100 cfu/g
yeast and mold[a]	<10 cfu/g	<10 cfu/g
Escherichia coli[a]	absent/g	absent/g
Salmonella[a]	absent/25 g	absent/25 g
Staphylococcus aureus[a]	absent/g	absent/g

[a]Each value was presented as an average of three measurements; *, ND not detected; $, not detected in the pesticides listed in the USFDA.

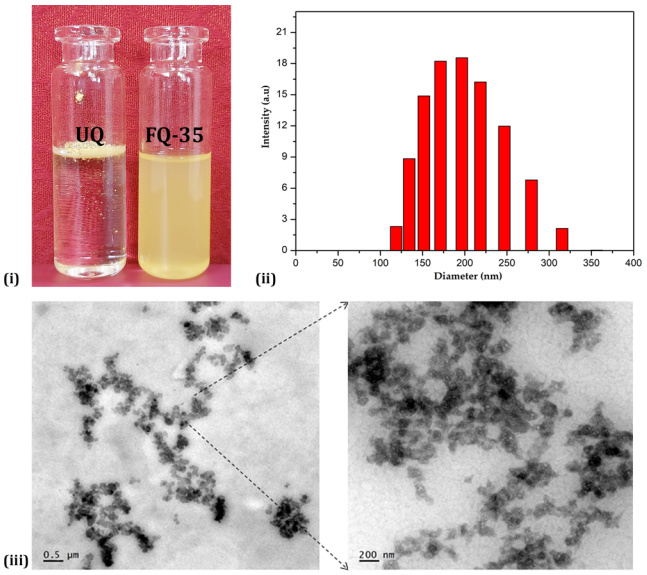

Figure 2. (i) Photograph of the aqueous solutions of UQ (left) and FQ-35 (right) indicating solubility. (ii) Hydrodynamic size distribution of FQ-35. (iii) TEM images of FQ-35 at different magnifications.

release of FQ-35. Figure 4 shows electron microscopic images of fenugreek galactomannan (FG) and the FQ-35, which showed an amorphous nature for FG and a nearly spherical-translucent morphology indicating the encapsulation and amorphous nature of FQ-35 [Figure 4(ii)].

The double-blinded randomized crossover study followed by ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) quantification of the quercetin content in plasma was performed. Ethyl acetate-based sample preparation and MRM transitions analysis (301.1 → 179.2; 301.1 → 151.1) showed a limit of quantification to be 1 ng/mL with a recovery of 90.6% (Figure 5A). This method exhibited linear range over a 1 to 1000 ng/mL concentration with an R^2 value of 0.9957 (Figure 5B). The method showed a reproducibility with an intra- and inter-assay precision of 4.8 and 7.5%, respectively. The accuracy of the method was found to be 92.9−102.6% upon intra-assay comparison and 91.2−104.3%, respectively, of the anticipated value upon inter-assay comparison. Matrix matched calibration was executed in the current study (Figure 5C). The quercetin-spiked and blank plasma samples showed no interfering peaks at the corresponding retention times of the analyte.

Both the UQ and FQ-35 were administered as identical 600 ± 10 mg capsules in which 90 ± 10 mg was the weight of empty hard-shell gelatin capsules. UQ contained 450 mg of quercetin with 98% purity (445 ± 5 mg), and each FQ-35 capsule was made up of 500 mg of FQ-35 powder with a 35.5% quercetin content (180 ± 5 mg). Therefore, all results shown were adjusted for the difference in quercetin content. The post-administration plasma concentration at different time intervals (time-course) implied that the plasma quercetin concentration following the consumption of FQ-35 was significantly higher ($P < 0.0001$) at all the post-administration time points (Figure 5B, inset). The average plasma concentration of quercetin was 62.08-fold higher (AUC_{0-24h} = 1703.50 ng·h/mL) for FQ-35 compared to that of UQ ($AUC_{0-24\ h}$ = 27.44 ng·h/mL). The C_{max} for quercetin was 314.66 ± 135.46 ng/mL at a t_{max} of 3.25 ± 0.44 h for FQ-35, compared to a C_{max} of 14.48 ± 6.65 ng/mL with a t_{max} of 1.12 ± 0.34 h for UQ. The $t_{1/2}$ was also significantly extended to 4.98 ± 0.51 h for FQ-35 while that of UQ was 1.91 ± 0.41 h (Table 2 and Figure 6). Moreover, all the 16 subjects completed the study without any significant side effect, so with respect to the safety conclusions, we can assert that the tested dose was well tolerated.

4. DISCUSSIONS

Quercetin, one of the most abundant dietary flavonoids, exhibits many health beneficial pharmacological effects including antioxidant, anti-inflammatory, anti-allergic, antiviral, anti-diabetic, neuroprotective, and anticancer effects.[31] However, the average intake of quercetin was estimated at about 15 mg/day (United States 9.7 mg/day, Spain 18.4 mg/day, Japan 15.5 mg/day),[18] which is demonstrated to be too low to elicit any favorable health benefits in clinical conditions because of its poor oral bioavailability, rapid biotransformation to glucuronides/sulfates, and fast elimination from systemic circulation.[32,33] A number of attempts to improve the oral bioavailability of quercetin by enhancing the solubility and gastrointestinal compatibility can be observed in the literature.[20,21,34,35] Nanostructured lipid carriers showed enhanced cytotoxicity and apoptosis.[36] Poly(ε-caprolactone)-*co*-D-α-tocopheryl polyethylene glycol succinate (PCL-TPGS) nanoparticles were reported with increased cytotoxicity.[37] Chen et al. developed a self-assembled lecithin-based mixed polymeric micelle delivery system to improve the solubility and bioavailability of quercetin.[38] A self-nanoemulsifying drug delivery system was reported to enhance the bioavailability of quercetin in rats.[39] Thus, micellar, liposomal, and self-emulsifying nanodelivery systems employing synthetic materials could be a plausible option to improve bioavailability, in spite of their disadvantages such as poor thermodynamic

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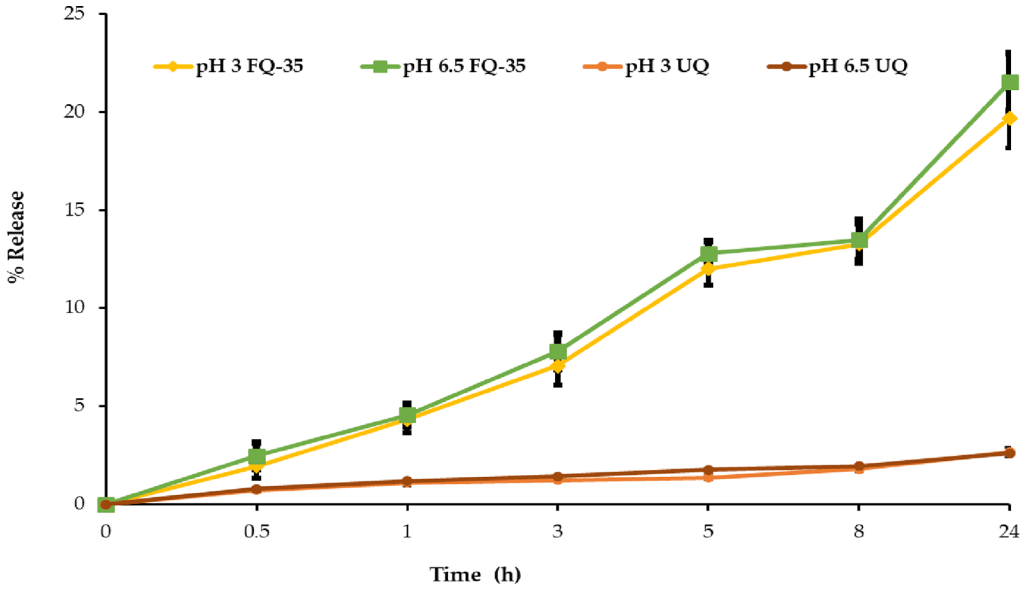

Figure 3. *In vitro* release profile of quercetin from FQ-35 and UQ at different pH.

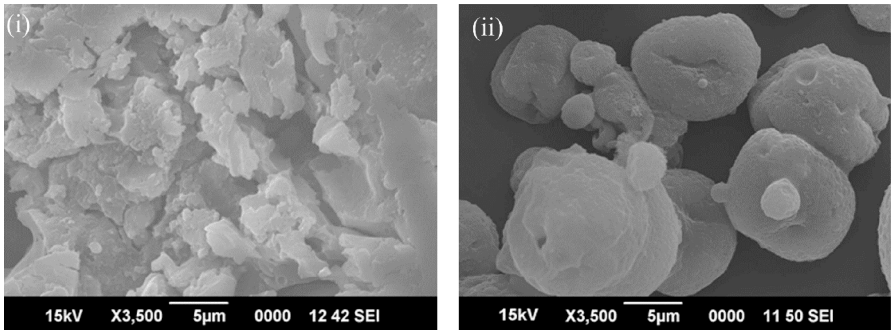

Figure 4. SEM images of (i) FG and (ii) FQ-35.

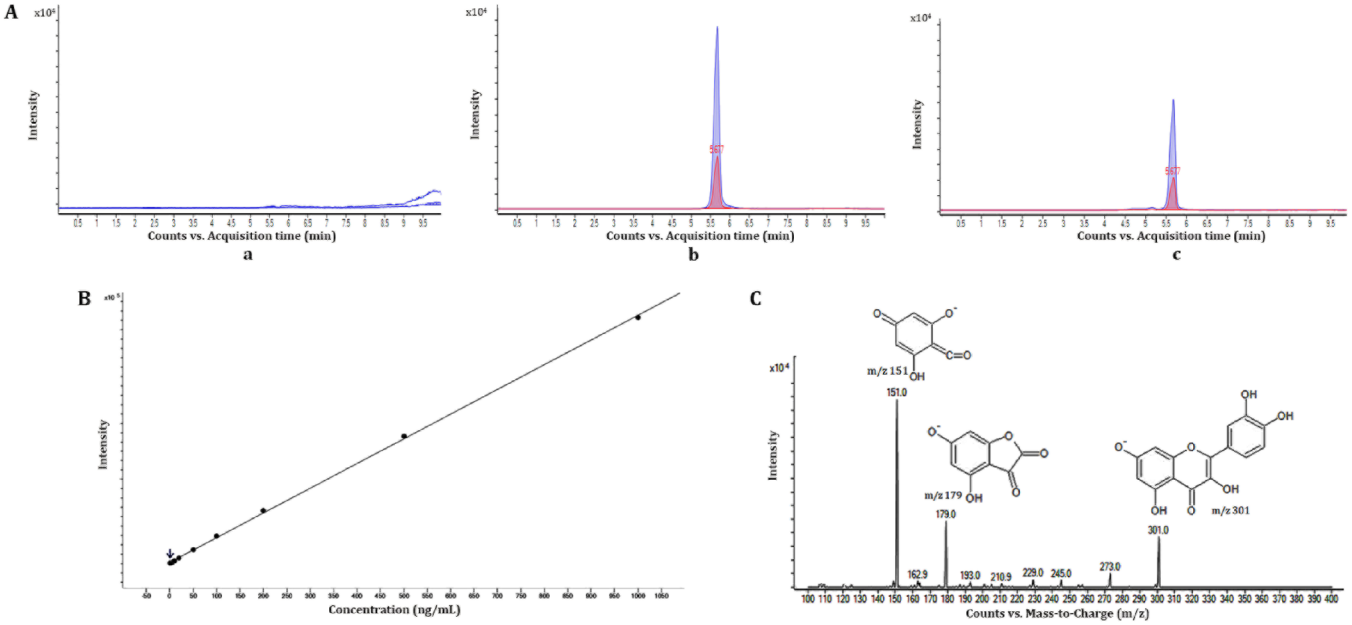

Figure 5. (A) UPLC-ESI-MS/MS analysis of quercetin in plasma. The MRM transitions yielded the MS/MS spectra with a signal/noise ratio >5.0 in (a) blank plasma, (b) standard quercetin, and (c) in plasma collected after 3 h of consumption of FQ-35. (B) Matrix-matched calibration curve of quercetin in plasma. (C) Mass spectra of quercetin *m*/*z* (301.1 → 179.2; 301.1 → 151.1).

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Table 2. Pharmacokinetic Parameters

sample	C_{max}(ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-24}(ng·h/mL)
FQ-35 (total)[a]	314.66 ± 135.46	3.25 ± 0.44	4.98 ± 0.51	1703.50 ± 348.67
FQ-35 (free)[a]	112.41 ± 52.30	3.38 ± 0.62	4.74 ± 0.51	510.86 ± 102.29
UQ	14.48 ± 6.65	1.12 ± 0.34	1.91 ± 0.41	27.44 ± 7.49

[a]"Total" represents the sum of free and conjugated metabolites of quercetin, and "free" represents the unconjugated quercetin content in plasma. C_{max}, maximum plasma concentration; t_{max}, time taken to reach the maximum concentration in plasma; $t_{1/2}$, time taken to reduce the plasma concentration to half of its maximum observed concentration; AUC, area under the curve.

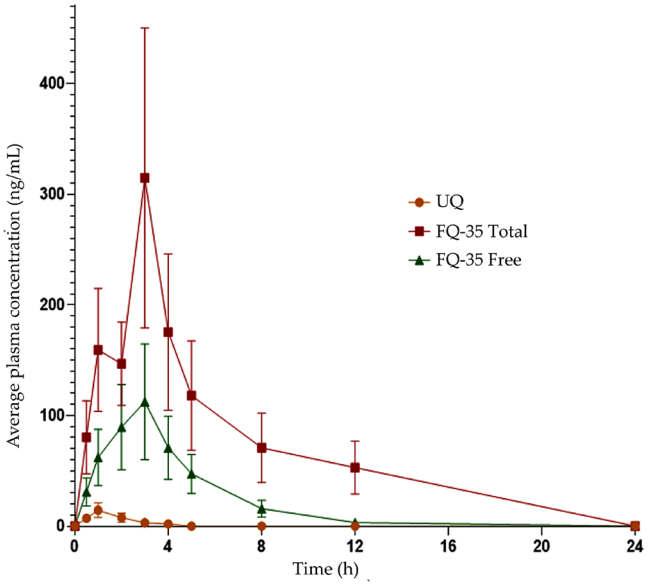

Figure 6. Average plasma concentration-time plot of free quercetin after oral administration of capsules. The total plasma quercetin content measured upon enzymatic hydrolysis versus time after ingestion of capsules. Statistical analysis was performed using SPSS software version 27, and all data points were presented as mean with standard deviation. The graph was plotted using Graph Pad Prism Version 5.0.

stability, poor stability under gastrointestinal conditions, low drug loading ability, liquid state, and excessive use of synthetic emulsifiers.[27,28]

The current study was aimed at the investigation of the morphological characteristics, solubility, *in vitro* release properties, and human pharmacokinetics of a novel food-grade hybrid-hydrogel formulation of quercetin (FQ-35), in comparison with unformulated quercetin (UQ). The formulation was prepared by a gel-phase dispersion of quercetin micelles into fenugreek galactomannan hydrogel (referred to as "Hybrid-FenuMAT") and further dehydration under vacuum to powder. Hybrid-hydrogels have been identified as a promising approach for oral drug delivery systems since they can enhance the *in vivo* stability and release properties of drugs.[40] High molecular weight, extensive hydration to stable softgel, mucoadhesive character, high emulsifying capacity, and amphiphilic nature to bind both oil and water make fenugreek galactomannan (FenuMAT) to behave like a cross-linked polymer for drug delivery.[27,28,41] The microstructure of FG allows the encapsulation of hydrophobic molecules in its conformationally directed hydrophobic pockets created in the swollen network. This is clear from the observed high encapsulation efficiency (>95%) of quercetin in FQ-35. The encapsulation of micellar quercetin into the hydrogel matrix to form FQ-35 powder was evident from the SEM image that shows the absence of the crystalline nature of quercetin but an amorphous and translucent morphology. Dynamic light scattering (DLS) and TEM analysis further confirmed the sub-micron size and spherical shape of FQ-35 particles in solution. The behavior of fenugreek galactomannan chains as a "hydrogel trap" for quercetin micelles is clear from the TEM image. Zeta potential, a measure of the stability of the suspended nanoparticles in solution, further demonstrated the stability of FQ-35 solution.

The pharmacokinetic study employing UPLC-ESI-QQQ-MS/MS to determine the plasma concentration of quercetin and its metabolites in healthy human volunteers was found to be in agreement with the low oral bioavailability of quercetin as previously reported by Riva et al.[30] Unformulated standard quercetin (UQ) provided a C_{max} of only 14 ng/mL, and no quercetin or quercetin metabolites were detected after 5 h of consumption. However, the plasma level of quercetin was significantly high during the 24 h post-administration time period for FQ-35, indicating its significant absorption and bioavailability. Area under the plasma concentration versus time curve estimation provided an 18.61-fold improvement in the bioavailability of free forms and 62.08-fold higher for total quercetin bioavailability compared to an equivalent dose of unformulated quercetin. The improvement in $t_{1/2}$ and t_{max} for FQ-35 supports the sustained intestinal delivery from the hydrogel scaffold.

The enhanced bioavailability and pharmacokinetic properties observed with FQ-35 may be attributed to its self-emulsifying capacity to enhance solubility, stabilized micellar structure, gastrointestinal compatibility, slow gastrointestinal transit, and the mucoadhesive property. The instant emulsification ability to disperse the nanoparticles when coming in contact with water or in the gastrointestinal tract makes FQ-35 a self-emulsifying hybrid-hydrogel with enhanced solubility (96-fold). Upon *in vitro* release analysis, FQ-35 was found to instantly disperse under gastrointestinal conditions and a slow release of solubilized and stabilized nanostructures was observed for improved absorption. Moreover, the mucoadhesive nature of fenugreek galactomannan may help to tightly bind to the microvilli and to overcome intestinal enzymatic biotransformation like glucuronidation. Such release properties of fenugreek galactomannan-based hydrogel formulations have already been reported for similar flavonoids, resveratrol, and fistein recently.[28,41] The hydrogel-quercetin system using β-lactoglobulin and sodium alginate has also been shown to exhibit good encapsulation efficacy and sustained release.[39] Similarly, Liu et al. have reported quercetin encapsulation on whey protein microgels coated with lotus root amylopectin and its enhanced in vivo bioavailability, indicating the viability of hydrogel delivery systems.[21]

Previously, it was also reported that the bioavailability of quercetin could be enhanced if fed with oils. The role of oil was ascribed to stimulate bile production and hence to

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improve the solubility of quercetin in lipid micelles. The enhanced solubility and oil miscibility can lead to a high concentration at the oil−aqueous interface for subsequent passage into the enterocytes.[42] So, the present formulation employed sunflower oil comprising phospholipids for the primary emulsification of quercetin into the hydrophobic−hydrophilic balance directed self-assembly to quercetin bilayer structures. In FQ-35, these lipid-based micelles were uniformly incorporated into the hydrophobic pockets created by the three-dimensional molecular network of soft hydrogel scaffolds. The hydrogel scaffold acted like a "trap" for the quercetin micelles and thereby supported the extended release of soluble nanoparticles of quercetin as evident from the *in vitro* studies.

5. CONCLUSIONS

Despite the chemical methods, green approaches for the oral delivery of phytonutrients with improved bioavailability are of great interest for phytonutrients and micronutrients for functional food and nutraceuticals. Herein, we demonstrated the preparation of a natural self-emulsifying reversible hybrid-hydrogel system for the oral delivery of quercetin with improved bioavailability and pharmacokinetic properties. The formulation (FQ-35) employed Hybrid-FenuMAT technology to engulf phospholipid-based micelles of quercetin within the conformationally restricted pockets of the fenugreek galactomannan hydrogel scaffold (FG) to form a hybrid-hydrogel followed by dehydration into powder such that it can instantly disperse in water or gastrointestinal fluid to form a soft hydrogel solution and hence to release the encapsulated quercetin molecules. The pharmacokinetic study on healthy volunteers further established the improved pharmacokinetics of FQ-35 compared to the standard unformulated quercetin (UQ). Area under the plasma concentration versus time curve estimation showed an 18.61-fold improvement in the bioavailability of free (unconjugated) forms and 62.08-fold higher for total quercetin bioavailability (sum of unconjugated and conjugated metabolites) compared to an equivalent dose of unformulated quercetin. Future studies are recommended on the in vivo effect of this enhanced bioavailable FQ-35.

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Notes

The authors declare the following competing financial interest(s): Hybrid-FENUMAT and QuerciFEN are the registered trademarks of Akay Natural Ingredients, Cochin, India who own the patent for the technology.

K.I.M. is the principal investigator who supervised the project and reviewed the manuscript. A.J. conducted the formulation development trials; A.B. validated the tandem mass spectrometry method; P.S. performed the characterization and original draft; B.M. reviewed the manuscript.

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