ORIGINAL ARTICLE

Quercetin encapsulated iron oxide nanoparticles with enhanced antioxidant and anticancer activities

K. Vijayasri[1], Ch. Alekhya[1], L. D. Srinivas[1], N. Anjali[1], M. Mathrusri Annapurna[2]

[1]Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy (Affiliated to Osmania University), Secunderabad, Telangana, India, [2]Department of Pharmaceutical Analysis, GITAM School of Pharmacy, Visakhapatnam, India

Abstract

The purpose of this study was to develop iron oxide nanoparticles loaded with quercetin that could effectively prevent cancer and exhibit antioxidant properties. Co-precipitation was employed to create the nanoparticles, which were subsequently evaluated for particle size, zeta potential, entrapment efficiency, and *in vitro* drug release. The optimized formulation, F5, was further characterized using techniques such as differential scanning calorimetry and scanning electron microscopy and tested for its anticancer and antioxidant activities, as well as intestinal penetration. The results showed that the addition of cyclodextrin and polyethylene glycol reduced the toxicity of ferrous oxide and improved the solubility of quercetin. The antioxidant activity of quercetin iron oxide nanoparticles was also demonstrated using H_2O_2 scavenging abilities. Furthermore, the drug permeation studies conducted on goat intestinal tissue showed that the F5 formulation had the highest levels of intestinal permeability, as well as anticancer activity and antioxidant properties, compared to the pure drug. In conclusion, the optimized quercetin iron oxide nanoparticles F5, with their optimal iron oxide, medium beta-cyclodextrin, and high surfactant levels, demonstrated improved drug release, antioxidant activity, and anticancer efficacy, making them a promising candidate for further development as a cancer prevention and treatment agent. The high scientific rigor and advanced methodology utilized in this study support its significance in the field of drug delivery and cancer research.

Key words: Anticancer activity and intestinal permeability studies, antioxidant, characterization, Iron oxide nanoparticles, *in vitro* drug release, Quercetin

INTRODUCTION

A biologically active flavonoid called quercetin (3,3′,4′,5,7-pentahydroxyflavone) [Figure 1] is abundant in edible fruits, vegetables, and medicinal plants such as apples, onions, and cabbage. Anti-inflammatory, antioxidant, anticancer, antiviral, and anti-ischemic properties are all exhibited by quercetin.[1-3] Nevertheless, it has low solubility in water, a brief biological half-life, and a limited oral bioavailability. As a result, the SPIONs[4] may be used to overcome these restrictions. Subsequently, quercetin was conjugated with SPIONs by Kumar *et al*. and Akal *et al*. Both research assessed the impact of quercetin conjugated with SPIONs on cells using an *in vitro* approach. Both studies used an *in vitro* method to evaluate the effect of quercetin conjugated with SPIONs on cells. The pharmacokinetic studies of quercetin have shown that poor absorption and rapid metabolism are the major reasons for the poor bioavailability of quercetin. Some of the possible ways to overcome these problems are discussed below. The dissolution rate of such a poorly water-soluble drug can be improved by increasing the particle surface area available for dissolution by reducing the particle size to nanoscale,[5-8] decreasing crystallinity or producing high energy amorphous form with a higher dissolution rate than use of a crystalline

Address for correspondence:
Dr. K. Vijayasri, Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy (Affiliated to Osmania University), Secunderabad, Telangana, India.
Phone:+91-9441341034.
E-mail: vijayasree_2002@yahoo.co.in

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form,[9] using an inclusion compound such as cyclodextrins.[10] Liposomes, phospholipid complexes, micelles, etc. are other promising novel drug delivery approaches,[11-13] which can provide higher solubility, better permeability, and resistance to metabolic processes for quercetin.

EXPERIMENTAL

Materials and reagents

Quercetin was purchased from Sisco Research Laboratories Ltd (MS). Iron oxide and Beta cyclodextrin were purchased from S D Fine Chem. Ltd., India. PLGA polymer was purchased from Sigma-Aldrich Mumbai. PEG-4000 was obtained from SRL Chemicals, Hyderabad. All other chemicals and reagents used were commercially available and of analytical grade.

The CaCo2 cancer cell line was purchased from NCCS, Pune, and the cells were maintained in DMEM medium supplemented with 10% FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2/95% air at 37°C.

Formulation of quercetin-loaded ferrous oxide nanoparticles

Iron oxide nanoparticles with quercetin loaded on them were created using the co-precipitation process [Figure 2]. After being dissolved in distilled water, iron oxide nanopowder is sonicated for 10 min. Iron oxide nanopowder solution is added, poloxamer 188 is dissolved in ethanol, and the mixture is agitated at 600 rpm for 5 h. Add cyclodextrin that has been dissolved in ethanol to the iron oxide poloxamer 188 solution and mix for 6 h at an 800 rpm speed. Iron oxide nanoparticles with cyclodextrin coating are created as a result. Filtered and baked in an oven. These nanoparticles were dispersed in water and quercetin dissolved in dimethyl sulfoxide was added to the solution and stirred at 6000rpm for 3 h which resulted in the formation of quercetin-loaded iron oxide nanoparticles. Thirteen different nanoparticle formulations were prepared and are shown in Table 1.[12].

Characterization of nanoparticles

Measurement of particle size and polydispersity index

Particle size of quercetin-loaded iron oxide nanoparticles is measured using Malvern particle size analyzer. Nanoparticles were diluted with distilled water and were analyzed for at least 3 times. The average values were employed for the calculations of the response surfaces.

Polydispersity index is the measure of the distribution of molecular mass in a given polymer sample.

$$\text{Polydispersity}=[D0.9-D0.1]\div D0.5$$

Where D0.9, D0.1, and D0.5 are particle diameters determined at the 90th, 50th, and 10th percentile of undesired particles, respectively. Small values of PDI indicate a homogeneous population, while high values of PDI indicate its high heterogeneity.

Measurement of zeta potential

Zeta potential shows the charge on the particle surface which indicates the physical stability of dispersed systems. The zeta potential of nanoparticles is measured using Malvern Zetasizer ZS 200. The zeta potential of a particle is the total innate charge acquired by it in a specific liquid medium. This

Table 1: Formulations of quercetin-loaded iron oxide nanoparticles

Formulation Codes	Iron oxide (mg)	Quercetin (mg)	Poloxamer (mL)	β-Cyclodextrin (mg)	DMSO (mL)	Ethanol (mL)
F-1	250	1000	4	100	2	1
F-2	250	1000	8	50	2	1
F-3	250	1000	4	100	2	1
F-4	250	1000	2	50	2	1
F-5	750	1000	2	25	2	1
F-6	750	1000	8	25	2	1
F-7	750	1000	4	50	2	1
F-8	750	1000	2	100	2	1
F-9	750	1000	8	100	2	1
F-10	500	1000	8	50	2	1
F-11	500	1000	2	50	2	1
F-12	500	1000	4	25	2	1
F-13	500	1000	8	100	2	1

technique measures the electrophoretic mobility exhibited by a charged particle which is directly proportional to its velocity, in a dispersion medium connected with a pair of electrodes. When a voltage is applied, this particle moves with a velocity toward the oppositely charged electrodes. Zeta potential was carried out on all 13 formulations. Briefly, 5 mg of nanoparticles were dispersed in 10 mL of distilled and sonicated for 5 min. The suspension was filtered through a syringe filter (1 mm cut off) to remove large aggregates, and the size of the nanoparticles was determined using the particle size analyzer. The zeta potential of the particles is a major factor in the stability of the dispersed particles. Hence, the zeta potential of the particles was calculated using the Delsa Nano C Zetasizer. Each sample for size and zeta potential was performed in triplicate and the results were expressed as mean value ± standard deviation (SD). Zeta potential is a physical property exhibited by all solid-liquid and liquid-liquid colloidal systems.

Surface morphology

The surface morphology of nanoparticles is measured by scanning electron microscopy (SEM) S-3700N. SEM is a test process that scans a sample with an electronic beam to produce a magnified image for analysis. During SEM analysis, the signals generated produce a two-dimensional image and reveal information about the sample, including chemical composition and external morphology.

Diffraction scanning calorimetry

Differential scanning calorimetry (DSC) studies were performed on pure drug and also optimized formulation. Samples were heated from 30 to 300°C at a heating rate of 10°/min in nitrogen atmosphere (flow rate, 20 mL/min) and the spectra were interpreted.

Evaluation of nanoparticles

Entrapment efficiency

Quercetin iron oxide nanoparticles are centrifuged at 10,000 rpm for 30 min using centrifuge. After centrifugation, supernatant is collected and the amount of unentrapped drug is determined using HPLC at UV 370 nm. The percentage entrapment efficiency is calculated by the equation

$$\%entrapment\ efficiency = \frac{Actual\ drug\ loading}{Theoritical\ drug\ loading}$$

In vitro dissolution studies

USP type II dissolution apparatus was used to measure the rate of release of Quercetin from the Quercetin-loaded iron oxide nanoparticles (in vitro) in phosphate buffer saline medium. 100 mg equivalent weight of quercetin-loaded iron oxide nanoparticles were suspended in 900 mL of phosphate-buffered saline (pH 6.8) maintained at 37°C. Five milliliters of the sample were picked up at regular time intervals for 24 h. Quercetin was estimated using HPLC at detection wavelength 370 nm. The cumulative percentage of quercetin release from quercetin-loaded iron oxide nanoparticles was calculated.

Statistical analysis

All drug diffusions were repeated 3 times and data were expressed as the mean±SD. Statistical data were analyzed by one-way ANOVA. A Dunnett's multiple comparison test was used to compare different formulations and $P < 0.05$ was considered to be significant.

Antioxidant activity

Antioxidant activity is defined "as an limitation of the oxidation of proteins, lipids, DNA, or other molecules that occur by blocking the propagation stage in oxidative chain reactions" and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction.

H_2O_2 scavenging activity

The procedure will be started with different concentrations (10–50 µg/mL) of test solution. 500 µL of buffer and 400 µL of 2 mM H_2O_2 were added. The mixture will be kept at room temperature for 5 min. After incubation, 2 mL of dichromate acetic acid reagent was added and color intensity was measured at 570 nm. The blank solution contains 2 mL of dichromate acetic acid alone whereas the reaction mixture without compound served as control. The percentage of inhibition will be calculated and compared with ascorbic acid as the standard.

Anticancer activity

3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The MTT cell proliferation assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. MTT assay is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. For MTT assay, each test compounds were weighed separately and dissolved in DMSO. For MTT assay, each of the test compounds were weighed separately and dissolved in DMSO and the made up to volume to get a final concentration of 1 mg/mL and then the cells were treated with a series of concentration of 10-100 µg/mL.

Cell viability was evaluated by the MTT assay with three independent experiments with six concentrations of compounds

in triplicates. Cells were trypsinized and perform the Trypan blue assay to know viable cells in cell suspension. Cells were counted by hemocytometer and seeded at density of 5.0 × 10.3 cells/well in 100 µL media in 96 well plate culture medium incubated overnight at 37°C. After incubation, take off the old media and add fresh media 100 µL with different concentrations of test compound in labeled wells in 96 plates. After 48 h, discard the drug solution and add the fresh media with MTT solution (0.5 mg/mL) to each well and plates were incubated at 37°C for 3 h. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and the concentration of test drug 30 needed to inhibit cell growth by 50% values is generated from the dose-response curves for each cell line using with origin software.

Intestinal permeation study

Freshly excised goat the duodenal part of the intestine was isolated and taken for the intestinal permeation studies. Then, this tissue was thoroughly washed with phosphate-buffered saline (pH 6.8) solution to remove the mucous and lumen contents. The quercetin-loaded iron oxide nanoparticles and quercetin (pure drug) sample were injected separately into intestinal mucosa and two sides were tightly closed. The receiver compartment was filled with 100 ml of phosphate-buffered saline pH 6.8 with continuous aeration and a constant temperature of 37°C. Mixing was performed by means of a magnetic stirrer at 50 rpm, and 1 mL samples were withdrawn periodically from the receiver compartment at time intervals of 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h diluted with 10 mL with phosphate-buffered solution and replaced with an equal volume of fresh transport medium. The absorbance was measured using a HPLC-UV detector at a wavelength of 370 nm, keeping the respective blanks.

RESULTS

The present study was aimed at the loading of the drug like quercetin encapsulated into iron oxide nanoparticles, to synergize the therapeutic activity of the drug.

Particle size determination and polydispersity index

The characterization of the mean particle size of quercetin-loaded nanoparticles is an important aspect of nanoparticle formulation for biomedical applications. The range of particle sizes observed between formulations (1173–4894 nm) indicates that there may be differences in the formulation parameters that are affecting particle size as shown in Table 2. However, the fact that the nanoparticles were uniform and

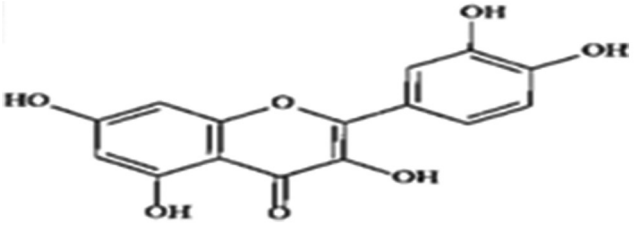

Figure 1: Chemical structure of quercetin

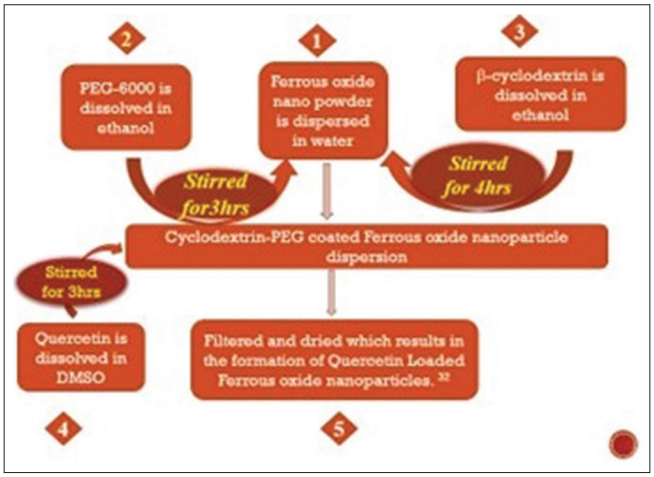

Figure 2: Preparation of quercetin-loaded iron oxide nanoparticles

monodisperse suggests that the particle size distribution within each formulation was narrow and consistent.

The smaller mean particle size observed for the F-5 formulation compared to the other formulations could be due to a variety of factors, such as differences in the composition of the nanoparticles, or the choice of surfactant or stabilizer as shown in Figure 3. The smaller size of the F-5 formulation could have potential advantages in increased bioavailability or enhanced cellular uptake due to improved penetration through cellular barriers.

The values indicated that the polydispersity index of all formulations of quercetin-loaded ferrous oxide nanoparticles (F-1 to F-13) varied from 0.317 to 0.892. These numbers suggest that the particle size distribution was largely consistent across all formulations. A narrow size distribution, or similar-sized particles in the sample, is often thought to be indicated by a PDI of <0.5. The statement also says that the F-5 formulation had a notably low PDI, indicating that the particles in this formulation were even more homogeneous in size than those in the other formulations. This shows that among all the formulations studied, the F-5 formulation may be the most stable and homogeneous.

Zeta potential

In general, nanoparticles with higher absolute values of zeta potential (either positive or negative) are more stable in

Table 2: Data for particle size, zeta potential, polydispersity index, and drug entrapment efficiency of quercetin-loaded ferrous oxide nanoparticles (F 1-F 13)

Formulation	Particle size	Zeta potential	Polydispersity index	Drug entrapment efficiency
F-1	4894	−23.5	0.545	70.04
F-2	3217	−20.1	0.715	63.7
F-3	2929	−22.5	0.690	71.1
F-4	1273	−18.4	0.892	71.40
F-5	1173	−19.0	0.637	77.9
F-6	1825	−15.5	0.695	65.8
F-7	1847	−23.9	0.637	66.5
F-8	3371	−20.5	0.600	72.4
F-9	2143	−26.4	0.394	69.3
F-10	2292	−23.0	0.484	62.5
F-11	2401	−14.9	0.384	68.7
F-12	4128	−20.9	0.556	69.9
F-13	2566	−21.7	0.317	64.3

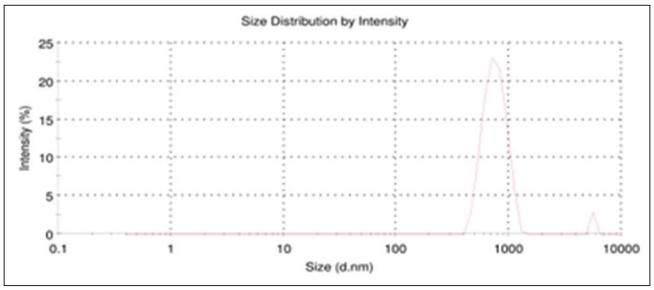

Figure 3: Particle size of Quercetin loaded iron oxide nano formulation F5

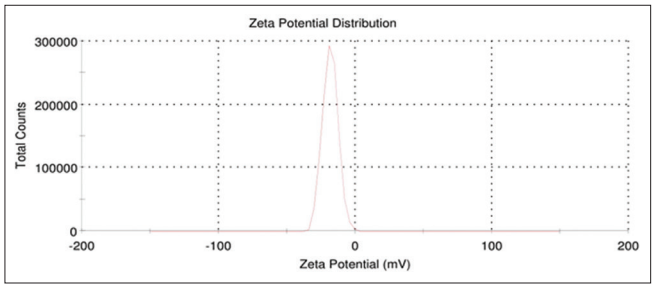

Figure 4: Zeta potential of Quercetin loaded iron oxide nano formulation F5

solution, as the repulsive forces between particles prevent them from aggregating and settling out of the suspension. As negative zeta potential values signify a greater number of negatively charged surface groups that repel one another and prevent particle aggregation, they are often linked to nanoparticles that are more stable in aqueous solutions. The zeta potential range (−10.6–−25.9 mV) for the nanoparticles in Table 2 indicates that they are moderately to very stable in solution. The zeta potential value for the F5 formulation was more negative as shown in Figure 4. It is crucial to remember, nevertheless, that the ideal zeta potential value range for a given application may change based on aspects including the size, shape, and surface chemistry of the nanoparticles, as well as the specific environmental conditions in which they will be used.

Entrapment efficiency

It appears that different formulations have varying degrees of quercetin-loaded iron oxide nanoparticle entrapment effectiveness. The size and surface characteristics of the nanoparticles, the technique of preparation, and the concentration of quercetin utilized in the formulation can all affect how much quercetin is entrapped as mentioned in Table 2. According to the data available, formulation F-5 had the highest entrapment efficiency of 77.40%, which indicates that a higher quantity of quercetin was really loaded into the nanoparticles throughout the preparation process.

In vitro dissolution studies

This experiment is likely investigating the potential for using quercetin-loaded iron oxide nanoparticles for drug delivery, specifically targeting absorption in the intestine. Iron oxide nanoparticles have been shown to have high surface area, which may allow for increased drug loading capacity and improved drug release. In addition, the magnetic properties of these nanoparticles may allow for targeted drug delivery to specific sites in the body. *In vitro* dissolution studies are a commonly used method to evaluate the rate and extent of drug release from a formulation. In this case, the studies were conducted on quercetin-loaded nanoformulations containing iron oxide, and the results are presented in Figure 5. The percentage of drug release was observed to increase within a range of 72.98–94.99%. This means that as time passed, more and more of the quercetin drug was released from the nanoformulations. The highest drug release, i.e., 95.99%, was

found for the formulation F5, indicating that this formulation released the drug more rapidly than the others. On the other hand, the lowest drug release of 72.98% was observed for the formulation F5, indicating that this formulation released the drug more slowly compared to the other formulations. The difference in drug release between formulations can be attributed to various factors such as the size and surface properties of the nanoparticles, the nature of the drug-polymer interaction, and the composition of the formulations. By analyzing the results of *in vitro* dissolution studies, researchers can optimize the formulation parameters to achieve desired drug release profiles for improved therapeutic efficacy.

SEM

The powerful imaging method known as SEM is used to investigate the surface morphology and structural characteristics of diverse materials. The spherical shape of the nanoparticles is likely due to the preparation method used, which can influence the final particle shape. The spherical shape and smooth surface of the formulation's nanoparticles, as shown in the SEM Figure 6, indicate that they are well formed and evenly dispersed. Although there is considerable size variation among the nanoparticles, the term "moderately uniform" denotes that overall, they are of a comparable size. The nanoparticles' smooth surface suggests that they are very homogeneous and devoid of any surface flaws or imperfections. The morphology and homogeneity of the quercetin nanoparticles in the formulation, which might affect their performance and usefulness in diverse applications, are both valuable insights provided by the SEM pictures.

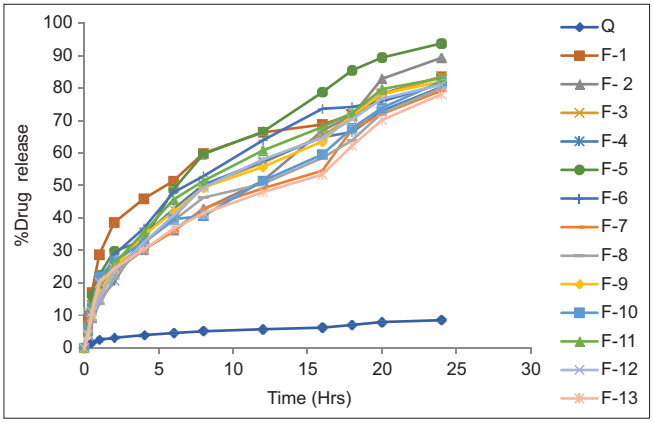

Figure 5: Dissolution studies for quercetin and Quercetin loaded iron oxide nano formulations (F-1 to F-13)

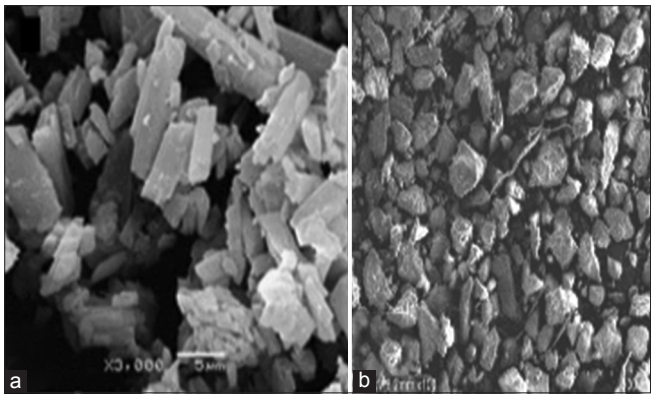

Figure 6: SEM of a) Pure Quercetin b) Quercetin loaded iron oxide nano formulation F5

DSC

The DSC thermogram of pure quercetin shows an exothermic peak at 313°C, which is likely due to the drug undergoing melting point. The DSC thermogram of quercetin the improved nanoformulation reveals an exothermic peak at a lower temperature of 182.38°C as shown in Figure 7. This lower temperature suggests that the melting point of the drug has been improved through the encapsulation within the nanoparticles. It is important to note that DSC is a thermal analysis technique that measures the heat flow associated with thermal transitions in a material.

Antioxidant activity

It appears that the study employed hydrogen peroxide (H_2O_2) scavenging activity as the method for evaluating the antioxidant activity of quercetin pure drug and quercetin iron oxide nanoparticles. It was found that quercetin nanoparticle concentration was higher than quercetin as a pure medication. Quercetin iron oxide nanoparticles and quercetin pure drug were both reported to have inhibitory percentages of 62 and 95, respectively, as shown in Table 3.

The higher inhibition % indicates that quercetin iron oxide nanoparticles had a better antioxidant activity than the pure quercetin drug, according to the highest concentrations. This might be caused by the iron oxide nanoparticles' intrinsic potential antioxidant properties or the increased quercetin concentration in the nanoparticles.

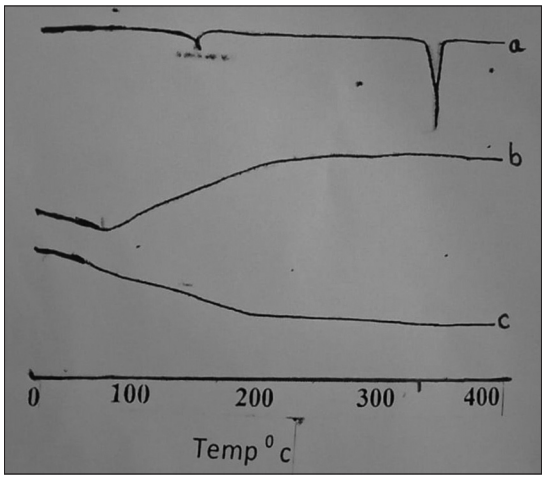

Figure 7: DSC Thermograms of a) Pure drug b) Iron oxide nanoparticles c) Quercetin loaded iron oxide nano formulation F5

Table 3: Antioxidant activity of quercetin and quercetin iron oxide NPs-F5

Conc (ug/mL)	% Inhibition of antioxidant activity	
	Quercetin	Quercetin iron oxideNPs-F5
0	0	0
10	25.9	29.9
20	32.5	50.4
30	40.5	71.3
40	48.3	81.9
50	55.4	95.7

Table 4: Percentage inhibition and % viability values of quercetin pure drug and of quercetin iron oxide NPs-F5

Conc (ug/mL)	Quercetin pure drug		Optimized Formulation-5	
	% Inhibition	% Viability	% Inhibition	% Viability
5	12.42	87.58	16.42	83.58
10	25.42	74.58	29.42	70.58
25	38.02	51.98	68.02	31.98
50	52.87	47.13	98.87	1.13

Anticancer activity

MTT assay

It appears that the study's main objective was to compare the cytotoxicity of pure quercetin and quercetin-loaded iron oxide nanoparticles on $Caco_2$ cells. Cell viability was assessed using the MTT assay after being exposed to various doses of the test chemicals in a 96-well plate as part of the experiment. Both the quercetin-loaded iron oxide nanoparticles and pure quercetin had their IC_{50} values (the amount of substance needed to inhibit cell growth by 50%) determined and shown in Table 4. The results suggest that when compared to the pure medication, the nanoparticles have a lower IC_{50} value like (57.59) (89.78). This suggests that at the studied concentrations, the nanoparticle has greater cytotoxic action against $Caco_2$ cells than pure quercetin. It is important to remember that this information only offers a basic comprehension of the study's methodology and conclusions. To completely evaluate the results, other information should be taken into account, such as the sample size, statistical analysis, and possible limitations.

Intestinal permeation study

Quercetin's absorption increased to 7.5 after 24 h, while quercetin-loaded nanoparticle F-5's absorption increased to 94. This shows that the iron oxide nanoparticles coated with quercetin may improve quercetin absorption in the gut. Quercetin-loaded iron oxide nanoparticles have been shown to have high surface area, which may allow for increased drug loading capacity and improved drug release [Figure 8]. Overall, this experiment provides initial evidence that quercetin-loaded iron oxide nanoparticles may enhance the absorption of quercetin in the intestine, which may have implications for drug delivery and absorption in humans. However, further studies are needed to fully evaluate the safety and efficacy of this approach.

Statistical analysis

All the experiments were performed in triplicate and the results were expressed as mean ± standard error (SE). SPSS software was used for data analysis and $P < 0.05$ was considered statistically significant.

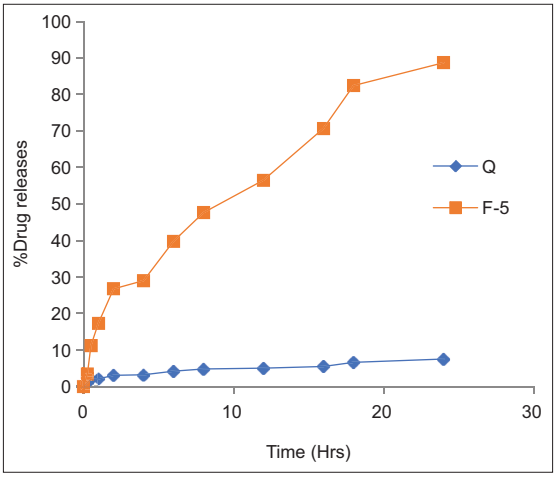

Figure 8: Intestinal Mucosal study of Quercetin and Quercetin loaded iron oxide nanoparticle F5

CONCLUSION

The magnetic nanoparticles possess powerful potential in medicine, covering both diagnostics and nano-based drug delivery. As versatile platforms, they can be simply functionalized for specific applications that benefit from their response to external magnetic fields. Quercetin is an active biological flavonoid found in large quantities in edible fruits, vegetables, and medicinal plants such as onions, cabbage, and apples. Quercetin has anti-inflammatory, antioxidant, anticancer, antiviral, and anti-ischemic effects. The bioavailability of the bioflavonoids is one of the most challenging aspects of formulation development. Superparamagnetic iron oxide nanoparticles with distinct properties such as a high surface-to-mass ratio, high magnetization, colloidal stability, cellular absorption, and biodistribution have emerged as the most effective parameters for transporting drugs, proteins, and probes. Nanoparticles were selected to improve the solubility, and ferrous oxide nanoparticles were selected to improve the bioavailability of the bioflavonoid quercetin. The ferrous oxide nanoparticulate system is one of the most successful developing areas in nanotechnology. The coating of these nanoparticles with polymers such as beta-cyclodextrin and polyethylene glycol reduces the toxicity of ferrous oxide and also has an

impact on the poorly soluble bioflavonoid quercetin. As the concentration of polyethylene glycol increased, particle size increased to some extent, and ferrous oxide nanoparticles also played an important role in reducing the particle size, as is evident from the particle size of batch F-5, which was 1173 nm. Out of all formulations, formulation F-5 has the highest entrapment efficiency of 77.40% when compared to other formulations. This is due to optimum polymer concentrations when compared to other formulations. The greater the entrapment efficiency, the greater the dissolution when compared to other formulations. *In vitro* dissolution studies for quercetin-loaded nanoformulations were also conducted, and the highest drug release, i.e., 93.71%, was found for the formulation F5. Antioxidant activity was performed using the H_2O_2-scavenging activity of quercetin pure drug and quercetin iron oxide nanoparticles. The antioxidant and anticancer activity of quercetin nanoparticles is higher when compared with quercetin as a pure drug. The drug permeation studies of the quercetin pure drug and the F5 formulation were conducted using goat intestinal tissue in an intestinal mucosal study. Quercetin-loaded iron oxide nanoparticles have been shown to have a high surface area, which may allow for increased drug loading capacity and improved drug release.

REFERENCES

1. Tian F, Dahmani FZ, Qiao J, Ni J, Xiong H, Liu T, *et al*. A targeted nanoplatform co-delivering chemotherapeutic and antiangiogenic drugs as a tool to reverse multidrug resistance in breast cancer. Acta Biomater 2018;75:398-412.
2. Aghapour F, Moghadamnia AA, Nicolini A, Kani SN, Barari L, Morakabati P, *et al*. Quercetin conjugated with silica nanoparticles inhibits tumor growth in MCF-7 breast cancer cell lines. Biochem Biophys Res Commun 2018;500:860-5.
3. Najafabadi RE, Kazemipour N, Esmaeili A, Beheshti S, Nazifi S. Using superparamagnetic iron oxide nanoparticles to enhance bioavailability of quercetin in the intact rat brain. BMC Pharmacol Toxicol 2018;19:59.
4. Najafabadi RE, Kazemipour N, Esmaeili A, Beheshti S, Nazifi S. Quercetin prevents body weight loss due to the using of superparamagnetic iron oxide nanoparticles in rat. Adv Biomed Res 2018;7:8.
5. Zhu B, Yu L, Yue QC. Co-delivery of vincristine and quercetin by nanocarriers for lymphoma combination chemotherapy. Biomed Pharmacother 2017;91:287-94.
6. Liu K, Chen W, Yang T, Wen B, Ding D, Keidar M, *et al*. Paclitaxel and quercetin nanoparticles co-loaded in microspheres to prolong retention time for pulmonary drug delivery. Int J Nanomedicine 2017;12:8239-55.
7. Ahmad N, Ahmad R, Naqvi AA, Alam MA, Ashafaq M, Rub RA, *et al*. Intranasal delivery of quercetin-loaded mucoadhesive nanoemulsion for treatment of cerebral ischaemia. Artif Cells Nanomed Biotechnol 2018;46:717-29.
8. Serri C, Quagliariello V, Iaffaioli RV, Fusco S, Botti G, Mayol L, *et al*. Combination therapy for the treatment of pancreatic cancer through hyaluronic acid-decorated nanoparticles loaded with quercetin and gemcitabine: A preliminary *in vitro* study. J Cell Physiol 2019;234:4959-69.
9. Sri KV, Kondaiah A, Ratna JV, Annapurna A. Preparation and characterization of quercetin and rutin cyclodextrin inclusion complexes. Drug Dev Ind Pharm 2007;33:245-53.
10. Daglioglu C. Tumour cell response to multidrug resistance with pH-sensitive quercetin and doxorubicin conjugated multifunctional nanoparticles. Colloids Surf B Biointerfaces 2017;156:175-85.
11. Ahmad N, Ahmad R, Naqvi AA, Alam MA, Rub RA, Ahmad FJ. Enhancement of quercetin oral bioavailability by self-nanoemulsifying drug delivery system and their quantification through ultra high performance liquid chromatography and mass spectrometry in cerebral ischemia. Drug Res (Stuttg) 2017;67:564-75.
12. Davatgaran-Taghipour Y, Masoomzadeh S, Farzaei MH, Bahramsoltani R, Karimi-Soureh Z, Rahimi R, *et al*. Polyphenol nanoformulations for cancer therapy: experimental evidence and clinical perspective. Int J Nanomedicine 2017;12:2689-702.
13. Farouk F, Abdelmageed M, Ansari MA, Azzazy HM. Synthesis of magnetic iron oxide nanoparticles using pulp and seed aqueous extract of *Citrullus colocynth* and evaluation of their antimicrobial activity. Biotechnol Lett 2020;42:231-40.

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