Original Article

Drug Bioavailability, Stability and Anticancer Effect of Berberine-loaded Magnetic Nanoparticles on MDA-MB-231 Cells in Breast Cancer

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ABSTRACT

Introduction: Breast cancer is the most causative factors of death in women. Treatment often consists of surgery, radiation therapy, and chemotherapy, have several side effects. Berberine is an alkaloid naturally-derived from *Berberis aristata* and a family *Berberidaceae* exhibits a broad spectrum of pharmacological benefits, including antiviral and anticancer properties etc. The recent development of nanomedicine is an art of delivering drugs to the target-site by improving their safety and efficacy. Among them, Magnetic nanoparticles play a key role to show their targeted drug delivery using a magnetic field. **Materials and Methods :** In this present study, we fabricated four berberine-loaded magnetic nanoparticles using a modified co-precipitation method with calcination. The resulting BBR/MNPs were characterized using FTIR, XRD, HRSEM, zeta potential, VSM, loading efficiency, stability, and *in vitro* release studies etc. The most proven MNP formulation type in dissolution was followed by *in-vitro* anticancer studies on MDA-MB-231 cells. **Results and Discussion:** XRD, FTIR and TGA results proved that the formed BBR/MNPs were ordered in their structure with iron, silanol groups and berberine moieties. HRSEM reported the average particle size of MNPs 100 to 250 nm after loading with berberine also had a regular spherical shape. The value of the zeta potential was -9 mv and 15 mv at p^H 6 for bare MNPs and BBR/MNPs, respectively. Loading efficiency and stability were good at BBR/MCM-41MNP. The saturated magnetization (Ms) value of Fe-MCM-41 MNP (81.76 emu/g) was obtained by VSM analysis. *In vitro* dissolution studies of four BBR/MNPs were reported at a three different P^H 5.5, 6.5, 7.4 including BBR/MCM-41 MNP were 86%, 84% and 82%, respectively. *In vitro* anticancer studies with BBR/MCM-41-MNP on treated MDA-MB-231breast cancer cells in comparison to standard Doxorubicin. The MTT assay confirmed the cytotoxic effect of BBR/MCM-41-MNP *in vitro*. The resulting data were statistically analyzed using one-way Anova analysis with $n=3$ replicates. The IC_{50} values (mean standard deviation) of BBR, BBR/MCM-41 MNP and standard doxorubicin were obtained as 16.754± 0.651, 6.750± 0.048, 4.955 ±0.042 μg/ml with significant $p<0.0001$. **Conclusion:** The best result was BBR/MCM-41 MNP with an average particle size 50 nm, which showed good drug loading efficiency and size stability above 7 days. Drug release was maximal (86%) at p^H5.5. The MTT-assay confirmed that BBR/MCM-41MNP exhibited more cytotoxicity on MDA-MB-231 cells than BBR, MCM-41MNP. The IC_{50} of BBR/MCM-41 was close that of standard Doxorubicin. BBR/MCM-41MNP showed optimum drug release with potent anticancer activity along with magnetic targeting.

Key words: Co-precipitation Method, FTIR, HRSEM, VSM, Loading efficiency, Stability, *In-vitro* release study, *In-vitro* anti-cancer study.

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INTRODUCTION

Globally, cancer is the second largest cause of death with an estimated 9.9 million deaths or one in six deaths, in the year 2020.[1] In men lung, prostate, colon, stomach and liver cancer were the most common cancer types. On the other side women were highly

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affected by breast, colon, lung, cervical, and thyroid cancers etc. Breast cancer is the commonest type of cancer in women, occurring 85% at the mucosal epithelium of the milk ducts and 15% at the lobules of glandular tissue in the breast.[2] The early stage of cancerous growth is limited to the ducts or lobules called *in situ*.[3-5] Upon time the *in situ* type cancers can develop into invasive breast cancer and then spread to regional metastases or surrounding organs in the body (distant metastases). In the 2020 global statistical year, 2,261,419 women were affected by cancer while 684,996 women died of cancer. In India, 3,465,951 women were morbid due to cancer and 1,121,413 women were mortal due to it. Treatment for breast cancer generally combines amputation, radiation therapy, and anti-cancer drugs (hormone therapy, chemotherapy, and/or targeted biological therapy) to treat the cancer. However, the treatment carries life-threatening side effects.[6-8] The anti-cancer activity can be evaluated both *in-vitro* and *in-vivo* in comparison to a standard drug on MDA-MB-231cells.

Nanoparticles are submicron ranged (100–1000 nm) microscopic particles that are usually composed of materials such as polymers, phospholipids and inorganic salts etc.[9] There are many kinds of Nanoparticles with varying size, shape, composition, and functionalities. They may contain liposomes, polymeric, iron salts, gold, quantum specks etc. Among the types of nanoparticles, magnetic nanoparticles are a specially designed system that delivers the drug to its desired site with the help of a magnetic field. The magnetic property was obtained by the core of the nanoparticle, which consists of metals such as cobalt, iron and nickel, etc.[10-12] Magnetic nanoparticles are attractive because they can load large amounts of drug molecules into their pores and eventually release them at the cellular level. Owing to their less toxicity, high stability and bio-compatibility, the iron oxide containing MNPs have unique clinical applications.

Natural herbal medicines have a multifaceted role in the prevention, diagnosis and treatment of ailments. During use, natural medicines show low side effects, low toxicity and more compatibility with all polymers used in the preparations. But it has some problems with low stability and low bioavailability. Here in this work has selected the drug called berberine. Berberine (BBR) belongs to isoquinoline alkaloids, extracted from various parts like root, rhizome, stem and bark of herbs such as *Berberis aristata* and *Berberis vulgaris*.[13-15] Active berberine components have shown diversified pharmacological uses, including antilipidemic, anti-depressant and anti-inflammatory effects, treatment of diabetics, and control of cardiovascular diseases, anticancer and antimicrobial properties.

In the present work, the fabrication of mesoporous magnetic particles was performed using a modified co-precipitation method. The formed magnetic nanoparticles were synthesized and confirmed by FTIR, XRD, and TGA to identify the optimal size, structural arrangement of the nanoparticles, and thermal stability of MNPs, respectively. HRSEM images of MNPs, zeta potential and VSM analysis were analyzed. All four MNPs were then measured for their percent drug release, stability, and colloidal potency. Finally, the optimized MNP efficiency was screened by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT assay on MDA-MB-231 treated cells and the percent cell viability and IC_{50} value with mean and SD were calculated. A Statistical study was performed by one-way Anova analysis using three numbers of replicates.

MATERIALS AND METHODS

Berberine in the form of pure powder was received as a complimentary sample from Himalayan Herbaria Inc., Uttar Pradesh, India. Pluronic P123 ($EO_{20}PO_{70}EO_{20}$), Tetra ethyl ortho silicate (TEOS, 98%), Conc. HCl (48%), Ethyl alcohol (>99.9%), Hexane and Butanol were procured from Merck labs. Cetyl trimethyl ammonium bromide (CTAB), Iron Acetyl acetone, and ammonium hydroxide (NH_4OH) was obtained from Sigma-Aldrich. All chemicals and polymers used were of analytical grade. MDA-MB-231 cell lines were obtained from Amala cancer Institute, Kerala, India.

Preparation Mesoporous Magnetic Nanoparticles BBR/SiO_2

Step 1: Synthesis of Sio2 mesoporous silica

SiO_2 mesoporous silica was prepared using a surfactant template, tri block co-polymer Pluronic P123 ($EO_{20}PO_{70}EO_{20}$).[16-17] A typical composition in mole ratio was TEOS: Pluronic P 123: Conc. HCl: H_2O: Ethanol (1:0.00967:0.0012:185:8.7) respectively. Place 1.4 g of P123 in 4.0 ml of ethanol. Weighed amount of 5.2 g of Tetra ethyl ortho silicate (TEOS) was added to the above reaction mixture. To this solution add 2.7 ml of 0.04 wt. % HCl and 6.0 ml of ethanol. The above mixture was stirred over night at 32°C. It was transferred to a Teflon bottle and heated and refluxed for 48 hr at 100°C. The resultant precipitate was filtered, washed with Double-deionized (DD) H_2O and then dried. The intact surfactant layer was removed by calcinating at about 500°C for 8 hr.

Step 2: Impregnation of Magnetite into Fe-SiO2 Using Iron (III) Acetate as a Precursor

0.3153 g of Iron (III) acetyl acetone dissolved in 3.0 ml of acetone with 0.3 ml of HNO_3 and stirred for 4 hr at 80°C. 0.25 g of SiO_2 particles were suspended in Fe $(AcetylAc)_3$ solution overnight and the solvent removed by stirring for 4 hr at 25°C. Then powder heated up to 500°C in a furnace for 2 hr with increasing temperature of 2°C per minute.

BBR/MCM-41

Step 1: Synthesis of (Mobil Composition of Matter No. 41) MCM-41 Mesoporous Silica

MCM-41mesoporous silica was prepared by placing 2.4 gm of Cetyl Trimethyl Ammonium Bromide (CTAB) in 120 ml of deionized water and stirred until the mixture was uniform and clear.[18-19] After adding 8 ml of ammonium hydroxide, the resultant mixture was stirred for 5 min, and then 10 ml of TEOS (98%) was added to the above surfactant solution and then stirred for 24 hr at RT. The so obtained white precipitate was washed with double-deionized (DD) H_2O, collected and then dried. By calcinating at 500°C for 8 hr, the surfactant template was removed.

Step 2: Impregnation of Magnetite into MCM-41 Using Iron (III) Acetate as a Precursor

0.3153 g of Iron (III) acetyl acetone dissolved in 3.0 ml of acetone with 0.3 ml of HNO_3 and stirred for 4 hr at 80°C. 0.25 g of MCM-14 particles were suspended in Fe(AcetylAc)3 solution overnight and the solvent removed by stirring for 4 hr at room temperature. Then powder heated up to 500°C in a furnace for 2 hr with increasing temperature of 2°C per minute.

BBR/KIT-6

Step 1: Synthesis of (Mobil Composition of Matter No. 41) KIT-6 Mesoporous Silica

KIT-6 mesoporous silica particles were synthesized using a tri-block co-polymer ($EO_{20}PO_{70}EO_{20}$) Pluronic P123 as the surfactant template. A typical composition in molar ratio found was TEOS: P123: HCl: H_2O: butanol (1.00: 0.017: 1.83: 195: 1.31) respectively. Take 1.23g of the surfactant Pluronic P123 in a mixture of 44 g of water and 2.25 g of Conc. HCl at 38°C-40°C. TEOS (98%) was then added to the above surfactant solution.[20-21] Then it was stirred for 20 hr at 32°C after transferring the above reaction mass to a Teflon bottle; it was heated to 100°C± 2°C for 48 hr. The so yielded white precipitate was filtered under vacuum, washed with double-deionized (DD) H_2O and air-dried. By heating to 500°C for 8 hr, the surfactant template was removed.

Step 2: Impregnation of Magnetite into KIT-6 Using Iron (III) Acetate as a Precursor

0.3153 g of Iron (III) acetyl acetone dissolved in 3.0 ml of acetone with 0.3 ml of HNO_3 and stirred for 4 hr at 80°C. 0.25 g of KIT-6 particles were suspended in Fe(AcetylAc)3 solution overnight and the solvent removed by stirring for 4 hr at room temperature. Then powder heated up to 500°C in a furnace for 2 hr with increasing temperature of 2°C per minute.

BBR/SBA-15

Step 1: Synthesis of SBA-15 (Santa Barbara Amorphous) Mesoporous Silica

SBA-15 mesoporous silica particles were developed using a tri block Pluronic P123 ($EO_{20}PO_{70}EO_{20}$) co-polymer as a template surfactant.[22] Molar ratio was found to be TEOS: P123: Conc. HCl: H_2O (1:0.017:2.9:202.6) respectively. To a mixture of water and Conc. HCl at 32°C±2°C, add the surfactant Pluronic P123. Add Tetra ethyl ortho silicate (98%) to the above surfactant solution, the mixture was stirred for 24 hr at 30°C±2°C. Transferred the resulting mixture into a Teflon bottle and heated to 100°C for 2 days. The white precipitate product was collected using filtration, washed with double-deionized H_2O and air-dried. By calcinating in air at 500°C for 8 hr, the attached reminant surfactant template was removed.

Step 2: Impregnation of Magnetite into SBA-15 Using Iron (III) Acetate as a Precursor

0.3153 g of Iron (III) acetyl acetone dissolved in 3.0 ml of acetone with 0.3 ml of conc. HNO_3 and stirred for 4 hr at 80°C. 0.25g of formed SBA-15 particles were placed in a Fe $(AcetylAc)_3$ solution overnight and the solvent removed by stirring for 4 hr at room temperature. Then powder heated up to 500°C in a furnace for 2 hr with increasing temperature of 2°C per minute. This sample was named as Fe–SBA-15.

Encapsulation of Drug into Magnetic Nanoparticles

0.4 g of the prepared mesoporous Fe-SiO_2, Fe-MCM-41, Fe-KIT-6 and Fe-SBA-15 samples were taken individually and added to 20 ml of a 1.4 g berberine-hexane solution and macerated for 3 days with stirring.[23] Then the berberine-loaded magnetic nanoparticles were obtained by centrifugation, collecting the product by filtration using hexane washing. Then the materials were dried at 60°C for 10 hr under vacuum.

Characterization of Berberine Loaded Magnetic Nanoparticles

Drug Response Study

Usually, UV−Visible spectrophotometer is an important for studying the formation of MNPs in aqueous

suspension. The graph includes BBR, BBR/SiO_2, BBR/MCM-41, BBR/KIT-6, and BBR/SBA-15. The UV−Vis spectrum of BBR and synthesized BBR-MNPs revealed that a new absorption peak at 403 nm.

FTIR

Fourier Transform Infrared Spectroscopy is employed to study the chemical composition of magnetic nanoparticles. The 0.2gm of sample were ground with 0.18gm of KBr powder and pressed to form a disc for FTIR scanning.[24] These pellets were analyzed in FTIR (prolific inst. Ltd, Mumbai, India). Then Data were collected at a wavenumber range of 400–4000 cm^{-1}.

X-ray Diffraction (XRD)

X-ray diffraction is used for the phase identification of crystalline materials as it provides information about average dimensions of nanocrystals. XRD (X-Ray Diffractometer) (schimadzu analytical Ltd, Maharashtra, India) was performed for understand the behavior of crystal structure of the synthesized Magnetic nanoparticles. The Scherrer formula expresses the volume weighted size of crystallites.

$$D = \frac{K\lambda}{B\cos\theta}$$

Where, D is the crystal size, λ is the radiation wavelength, β is the physical width of a reflection (in 2θ), θ_0 is the diffraction angle of a line maximum, and K is a constant.

Thermal Gravimetric Analysis (TGA)

Thermal Gravimetric Analysis (TGA) is performed in a nitrogen atmosphere using the SDTQ 600 instrument. The sample was placed in an aluminum pan and heated from room temperature (25°C) to 250°C at a heating rate of 10°C/min.

High Resolution Scanning Electron Microscopy (HRSEM)

Measurement of average diameter of nanoparticles was performed in de-ionized water by the dynamic light scattering technology at room temperature (25°C) and morphology features of polymeric nanoparticles were studied using a scanning electron microscopy (SEM instrument S-4800).[25]

Loading Efficiency

UV–Visible spectroscopy is used to estimate the content of Berberine (Drug) at a wavelength of 403 nm. It is used to calculate the drug loading.

$$\text{Drug Loading Efficiency} = \frac{(W_0 - W_1)}{W_{NP}} \times 100$$

Where, W_0, W_1, and W_{NP} represent the initial weight of Berberine, the weight of Berberine in the solution, and weight of the BBR-MNPs respectively.

Colloidal Stability of Berberine Magnetic Nano particles

The stability of Berberine loaded Magnetic nanoparticles was determined by colloidal stability analysis, it was conducted for seven days using DLS at 37°C which mimics the physiological conditions. Samples were prepared with deionized water with an adjusted concentration at 1mg/ml. Then it was used to determine the colloidal stability of the particles.

$$\text{Colloidal Stability } (t_n) = \frac{\text{Nanoparticle Size } t_n}{\text{Initial Nanomparticle size } t_0} \times 100$$

Where, colloidal stability of the particles in each day (t_n) is equaled to the nanoparticle size of each day (t_n) to the initial size of the nanoparticle at the first test (t_0).

Zeta potential (ζ)

Measurement of Zeta potential was identified by Zetameter. The zeta potential is the degree of repulsion between adjacent, similarly charged particles in any dispersion. Generally, an optimal zeta potential with small particle size decides the stability.[26]

Vibrating Sample Magnetometer Analysis

Vibrating Sample Magnetometer (VSM model) instrument used to estimate the magnetic properties of Fe-MCM-41Magnetic Nanoparticles. The hysteresis curve obtained between applied magnetic field from -20000 to 20000 Oe on X-axis and Magnetization (emu/g) on Y-axis.

In-vitro Dissolution Study

Three different dissolution media such as phosphate buffer solutions (PBS) p^H 5.5, pH 6.5 and pH 7.4 were selected for *in-vitro* dissolution studies.[27] According to the United States Pharmacopeia (USP) Method II (the paddle method), the bath volume for each medium is 900ml at 37°C±0.5°C and its rotational speed is 100 rpm.[24] Placed Crude BBR (100 mg) and prepared BBR loaded MNPs into the dissolution vessels. 5 ml of sample aliquot was removed at predetermined time intervals (i.e. 2 hr, 4 hr, 8 hr, etc. up to 36 hr) and filtered using Whatman filter paper No. 1. To maintain the sink conditions, same volume of fresh medium was replaced. The filtered samples were appropriately diluted and measured for their absorbance using a dual-beam spectrophotometer (Lab India UV/Visible Spectrophotometer, India) at a maximum wavelength of 403 nm. All test data were tabulated clearly.

$$\% \text{ Amount of drug released(mg/ml)} = \frac{\text{Conc} \times \text{Bath Vol.} \times \text{dil. factor}}{1000} \times 100$$

In-vitro Cytotoxicity Studies of Berberine loaded MCM-41 MNPs

The Cytotoxicity Study of Berberine loaded MCM-41 MNPs was performed on selected Breast Cancer Cells named MDA-MB-231 determined by Cell Proliferation Assay with MTT Reagent.[27-28] The cytotoxic effects of free MNP MCM-41, BBR and BBR/MCM-41 were evaluated on breast cancer cells i.e., MDA-MB-231 using 3-(4, 5-dimethylthialzol-2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) assay methods.[25] The MDA-MB-231 cells were treated by free MNP MCM-41, BBR and BBR/MCM-41 at different concentrations ranging from 0−40 µg/ml for 24 hr. The MDA-MB-231 cells were placed in 96-well plates (5000 cells/well) and cultured with free medium, following which the cells were treated with 0, 2, 5, 10, 20, 40, 80,100µg/ml of free MNP MCM-41, BBR, BBR/MCM-41 and Doxorubicin Standard for 24 hr at 37°C. Then the medium was discarded and the cells were treated with 0.1% MTT reagent and incubated for 4 hr at 37°C. The formazan crystals so formed were solubilized in dimethyl sulfoxide (DMSO) and the absorbance was noted immediately at 496 nm using a micro plate reader. Equivalent amounts of dimethyl sulfoxide (DMSO) were served as control for this experiment. The principle of MTT assay relates to quantifying the conversion of the tetrazolium compound into the formazan product using metabolically active viable cells.

$$\% \text{ cell viability} = \left[1 - \left(\frac{\text{No. of purple colored cells}}{\text{No. of total cells}}\right) \times 100\right]$$

The percentage of inhibition of cell proliferation was calculated using the equation. IC_{50} is defined as the concentration required reducing the 100% cells to 50%. IC_{50} value was calculated and plotted curves using the below equation.

$$\% \text{ cell growth inhibition} = \frac{(\text{Abs zero} - \text{Abs sample})}{\text{Abs zero}} \times 100$$

RESULTS AND DISCUSSION

Drug Response Study

The prepared BBR/MNPs were characterized, evaluated for Drug response study by UV/Vis spectroscopy, reported to have a strong absorption band at 251 and 430 nm, which can be attributed to charge transfer and the extra framework of iron clusters on the silicas. Figure 1 showed absorption peaks at 232nm, 266nm, 346nm, 403nm and 428 nm of all the four formulations

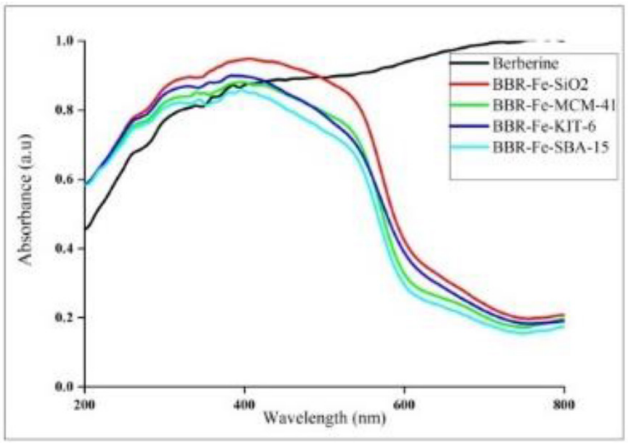

Figure 1: Drug Response Study.

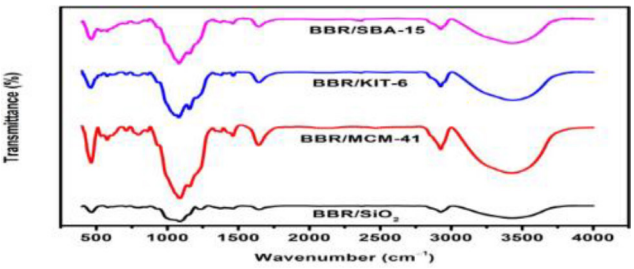

Figure 2: FTIR spectrum of berberine loaded MNPs.

and Berberine respectively. In the present study, a broad peak was observed at 210-607 nm which can be assigned to the overlap of the Fe_2O_3 and BBR absorption bands. This result confirmed the surface interaction of iron nanoparticles and drug on the surface of the silica materials.

FTIR

The BBR drug exhibited a characteristic band located at 3410 cm^{-1} representing O−H stretching vibrations explaining the presence of water molecules on all materials. Other major bands expressed at 2922 cm^{-1} and 2853 cm^{-1} indicated C−H stretches (alkanes). Similarly, peaks at 1646 cm^{-1}, 1140 cm^{-1}, and 1157 cm^{-1} correspond to aromatic C=C vibrations, C-H bending in-plane and C−H vibrations respectively. These major peaks were in line with the earlier report. In addition, two peaks at 577 cm^{-1} and 709 cm^{-1} are attributed to stretching and bending vibration modes of the Fe–O in the Fe–O–Si bonds in (Figure 2).

X-ray diffraction (XRD)

The peaks observed at 24.3°, 33.2°, 35.7°, 49.6°, 54.2°, 62.6° and 64.2° are due to Fe_2O_3 on the surface of the silica materials. The peaks observed at 14.9 °, 18.2 °, 41.0 ° are due to characteristic peaks of drug berberine present in all materials (Figure 3). These results were

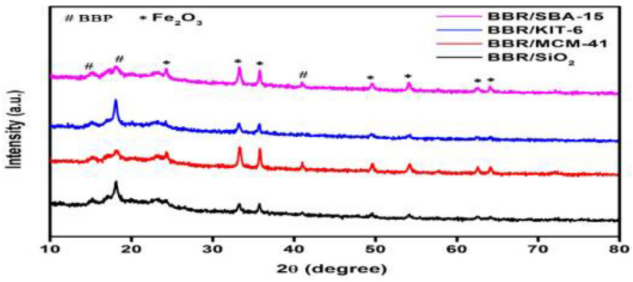

Figure 3: XRD curves of berberine loaded MNPs.

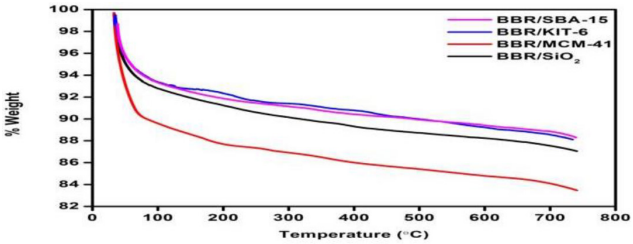

Figure 4: TGA of BBR loaded MNPs.

consistent with previously reported results. These results indicated the successful loading of drug on all the prepared Magnetic Nanoparticles (Fe-SiO_2, Fe-MCM-41, Fe-KIT-6 and Fe-SBA-15).

Thermal Gravimetric Analysis (TGA)

TGA profiles of all materials shown in the Figure 4 were reported showing two stages of weight loss observed at different temperatures. The initial weight loss observed below 200°C can be understood as due to the removal of absorbed water from the material and the decomposition of drug from the material. The BBR drug has been reported to degrade between 200 and 250°C (Figure 4). The weight loss between 250°C and 750°C is due to dehydroxylation from the surface silanol group (Si-OH). Weight loss appears to be less with SBA-15 compared to all materials.

Scanning Electron Microscopy (HRSEM) Images

HRSEM images revealed that all the materials possess irregular shape, spherical like morphology which is due to the drug loading on the surface of the silica materials containing Nanoparticles. SEM graphs of all MNPs before and after drug loading increased their diameter indicated clearly, and its size range from 100nm – 250nm. Among all types of MNPs, BBR/MCM-41 and BBR/SBA-15 possessed optimum size range with good appearance (Figure 5).

Loading Efficiency

Loading efficiency of all four Berberine loaded MNPs were calculated. As Figure 6 indicated, percentage of

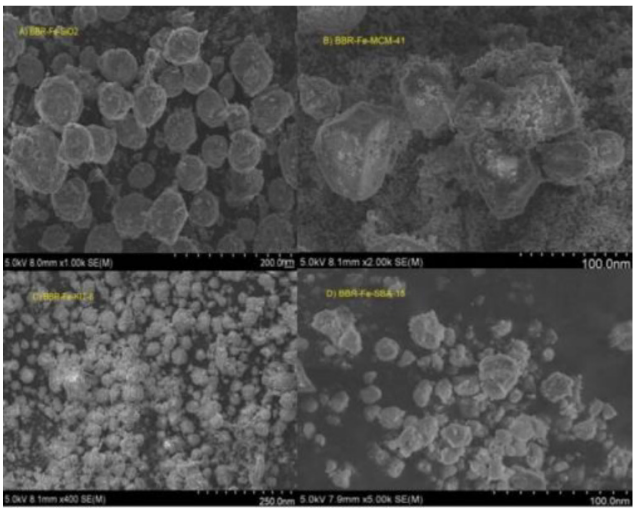

Figure 5: HRSEM Images of BBR loaded MNPs, A) BBR/SiO2 B) BBR/MCM-41 C) BBR/KIT-6 D) BBR/SBA-15.

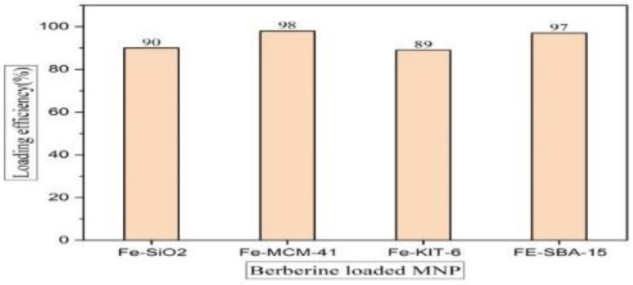

Figure 6: Drug Loading Efficiency of formed MNPs.

drug loading was high in BBR/MCM-41 with 98%. The other three MNPs drug loading percentages were also in the range of 90-95%.

Colloidal Stability

Colloidal stability analysis was conducted for seven days using Dynamic Light Scattering instrument at 37°C and nanoparticles size was determined. The percentage stability of Berberine loaded Magnetic nanoparticles was shown in the Figure 7.

Zeta potential (ζ)

The zeta potential values at P^H 3 to 11 for the electrostatic stabilization of NPs were estimated. This indicated surplus negative charge of Fe-MCM-41 MNP which might be due to the presence of Iron molecules on their surface. Figure 8 shows that the final product, BBR/MCM-41, possessed a positive charge due to presence of Drug named Berberine on its surface.

Vibrating Sample Magnetometer Analysis

VSM gives the hysteresis curves for the magnetic nanoparticles. Among all four Magnetic Nanoparticles,

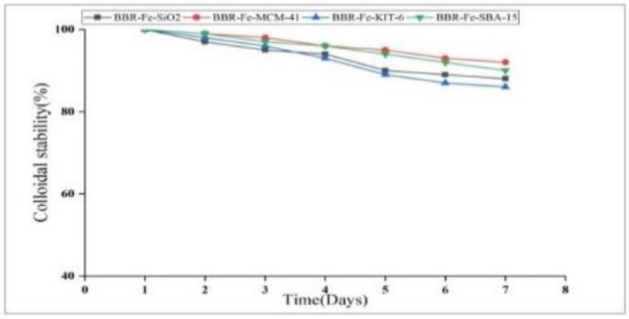

Figure 7: Colloidal Stability of loaded MNPs.

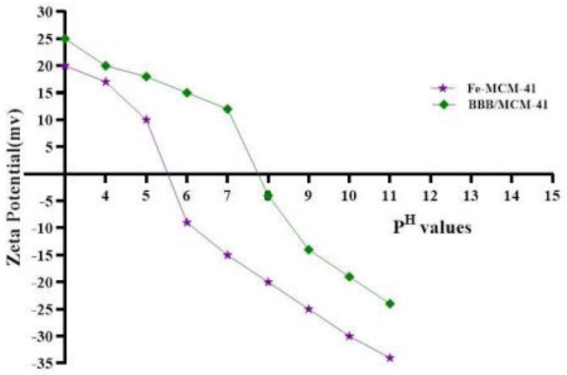

Figure 8: Zeta potential study of Fe-MCM-41 and BBR/MCM-41 MNPs.

Fe-MCM-41 MNPs before drug loading possessed an optimum particle size i.e., 50 nm. Then, the VSM study was done for the Fe-MCM-41 MNP. The magnetization property of synthesized Fe-MCM-41 Magnetic nanoparticles was analyzed at 37°C (normal human body temperature). The saturated magnetization (Ms) value of Fe-MCM-41 MNP was 81.76emu/g, as obtained by Vibrating Sample Magnetometer (VSM) analyses. Zero remanence and coercivity was observed in the hysteresis loops, indicating that the synthesized Fe-MCM-41MNP was superparamagnetic. (Super-paramagnetic materials can be easily magnetized when exposed to a magnetic field and can be unmagnetized, when the induced magnetic field is turned off). The prepared Fe-MCM-41 magnetic nanoparticles have a small core size i.e., 50 nm, has shown a magnetic behaviour with zero remanence and coercivity values given in Figure 9. The synthesized MNPs in this study were proved to be super-paramagnetic. This property is a desired characteristic in bio-medical applications for several diseases. These magnetite Fe-MCM-41 Magnetic nanoparticles, stabilized by the loading with drug named Berberine. Loaded Magnetic nanoparticles can be effectively used for diagnosis, imaging and for therapy owing to their low particle size with high surface area to volume ratio.

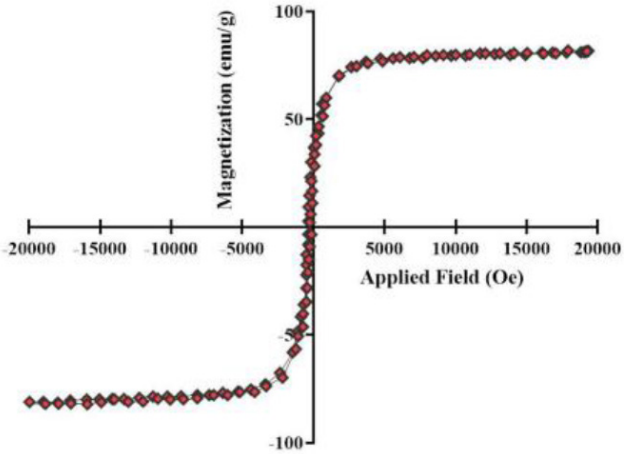

Figure 9: Vibrating Sample Magnetometer analysis for Fe-MCM-41 MNP.

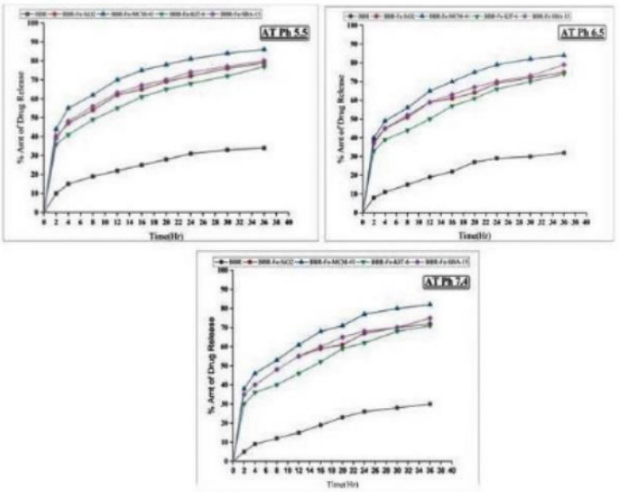

Figure 10: *In vitro* dissolution graph of BBR MNPs.

In-vitro Dissolution Study

In-vitro Dissolution studies of all four types of MNPs at three different P^H 5.5, 6.5 and 7.4 were reported in Figure 10. At P^H 5.5, 6.5, 7.4 the % drug release of pure BBR results were 34± 0.71, 32± 1.03, 30± 1.31 respectively. Among all the four MNPs, BBR/MCM-41MNP is the only formulation meeting the prerequisites of a Prototype MNP. The proved formulation BBR/MCM-41MNP's dissolution carried using Phosphate Buffer solutions at P^H 5.5, 6.5, 7.4 exhibited 86%, 84%, 82% of drug release after 36hr. This results indicated that the drug release was greater for BBR loaded MNPs than the pure BBR due to its Nano sized particles with more surface area, which leads to more bioavailability. As the P^H decreases, the % of Drug release from MNPs increases. So, acidic P^H conditions are favorable.

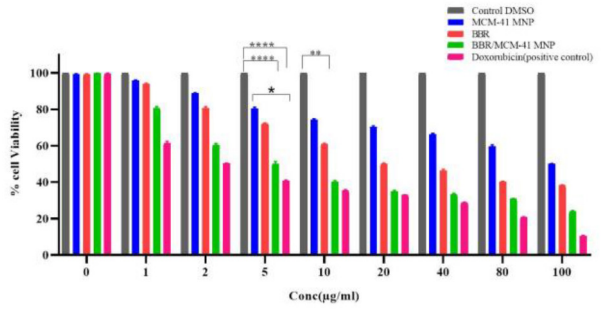

Figure 11a: % Cell viability by MTT assay. The % cell viability of control DMSO, BBR, MCM-41 MNP, BBR/MCM-41 MNP and Std Doxorubicin on treated MDA-MB 231 breast cancer cell lines at 24hr. Statistical analysis was performed using multiple variance analysis (one way ANOVA) (*p<0.01, **p<0.01, ****p<0.0001, significant and n=3).

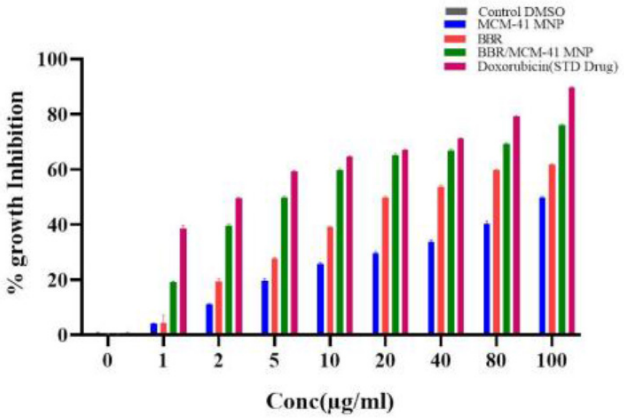

Figure 11b: % Cell growth Inhibition calculated for control DMSO, BBR, MCM-41 MNP, BBR/MCM-41 MNP and Std Doxorubicin on treated MDA-MB 231 breast cancer cell lines with mean ± SD.

In-vitro Cytotoxicity Study of Berberine Loaded MCM-41 MNPs

It is essential to evaluate the cytotoxic nature of formulated BBR/MCM-41 MNP for their anti-cancer benefit(s). The cytotoxicity potential was determined by MTT assay method. In our *in-vitro* cytotoxicity study, Standard drug employed was Doxorubicin. The cytotoxic effects of standard Doxorubicin, BBR, MCM-41 MNP and BBR/MCM-41 MNP were tested on breast cancer cells lines i.e., MDA-MB-231 cells at 24 hr incubation time. The MDA-MB-231 cells were treated to varying concentrations ranging from 0-100 µg/ml of standard Doxorubicin, BBR, MCM-41 MNP and BBR/MCM-41 MNP for 24 hr. In case of BBR treated cancer cells, the viability of the cancer cells decreased sharply as concentration increases (0- 100µg/ml) (Figure 11A). A similar result was seen with BBR/MCM-41 MNP at all tested concentrations. The MTT assay values exhibited a concentration based proliferative effect (0–100 µg/ml) by BBR and BBR/MCM-41 MNP formulations as seen in the study of % cell viability. Our prepared BBR/MCM-41 MNP compared with selected standard drug Doxorubicin was significant p< 0.0104 at multiple variance analysis (one way ANOVA). IC_{50} value is the exact half inhibitory concentration (50% cell growth inhibitory concentration), it was found to be, (Figure 11B) 16.754± 0.651 with BBR, 6.750± 0.048 with BBR/MCM-41 MNP, 4.955 ±0.042 with Doxorubicin (STD Drug) treatment. In the cell growth inhibitory study, our prepared BBR/MCM-41 MNP compared with selected standard drug Doxorubicin was significant p< 0.0027 at multiple variance analysis (one way ANOVA). Table 1 noted that the BBR/MCM-41 MNP exhibited good inhibition upon MDA-MB-231 cells growth. Due to the targeted drug delivery, the increased inhibitory rate effected cell viability as shown in Figure 11C. Using multiple variance analysis (one way ANOVA), the statistical analysis was performed with three number of replicates.

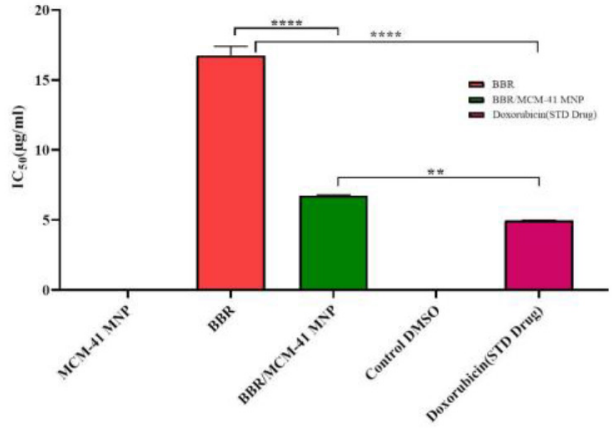

Figure 11c: IC_{50} value calculated for control DMSO, BBR, MCM-41 MNP, BBR/MCM-41 MNP and Std Doxorubicin on treated MDA-MB 231 breast cancer cell lines. Statistical analysis was performed using multiple variance analysis (one way ANOVA) (p<0.02 and ****p<0.0001, significant and n=3).**

Table 1: IC_{50} value by Mean ± SD Values.

BBR	BBR/MCM-41 MNP	Doxorubicin (STD Drug)
16.754± 0.651	6.750±0.048	4.955 ±0.042

CONCLUSION

In the present study, four Berberine-loaded magnetic nanoparticles were prepared using modified co-precipitation methods with calcination. Then they were characterized methods such as Drug Response Study, FTIR, XRD, TGA, HRSEM, Loading Efficiency,

Colloidal Stability, Zeta Potential, VSM, *in-vitro* Drug Release Study against time and *in-vitro* Cell line studies. The Drug Response Study found that berberine had a maximum absorption at 403 nm. The resulting XRD showed that the formed composites retained an ordered mesoporous structure after the formation of iron oxide nanoparticles in the pores. FTIR indicated that the surface contains silanol groups and Fe-O on the surface of the materials at 1093 cm^{-1} and 1020 cm^{-1} peaks, respectively. TGA indicated that BBR/KIT-6 MNPs was little more stable at different temperatures as it has shown less weight loss. Scanning electron microscopy (SEM) revealed that all the mesoporous magnetic nanoparticles are within the size range of 50 nm to 200 nm before loading and 100 to 250 nm after loading with Berberine and also have a regular spherical shape. All four BBR contained MNPs with good loading efficiency and colloidal stability. The zeta potential of Fe-MCM-41 MNP was negatively charged; suggesting that the excess negative charge of Fe-MCM-41 NP could be due to the presence of iron molecules on its surface. The final product, BBR/MCM-41 MNP nanomaterial possesses a positive charge due to its presence of drug named Berberine on its surface. The saturated magnetization (Ms) value of Fe-MCM-41 MNP (Iron oxide MNP without drug) (81.76 emu/g) was obtained by VSM analyses. *In-vitro* Dissolution study of all four types of MNPs at three different P^H 5.5, 6.5, 7.4 were reported, among all BBR/MCM-41MNP at three different P^H 5.5, 6.5, 7.4 exhibited 86%, 84%, 82%. The synthesized Magnetic nanoparticles revealed potential anticancer activity tested in *in-vitro* breast cancer cell lines i.e., MDA-MB 231 cells evaluated by means of MTT assay. The percentage cell viability and IC_{50} values of BBR, BBR/ MCM-41 MNP and Doxorubicin Standard drug was calculated to 16.754± 0.651, 6.750± 0.048, 4.955 ±0.042 respectively. The all the data was obtained statistically with One-Way Anova analysis at mean and SD with three number of replicates.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MNP: Magnetic Nanoparticle; **TEOS:** Tetra ethyl ortho silicate; **CTAB:** Cetyl Trimethyl Ammonium Bromide; **SEM:** Scanning Electron Microscope; **ml:** Milli Liter; **°C:** Degree Centigrade; µg: Microgram; **rpm:** Revolutions per minute; µm: Micrometer; **nm:** Nanometer; **MCM-41:** Mobil Composition of Matter No. 4; **KIT-6:** Mobil Composition of Matter; **SBA-15:** Santa Barbara Amorphous; **SiO_2**: Silicon Oxide. **BBR:** Berberine.

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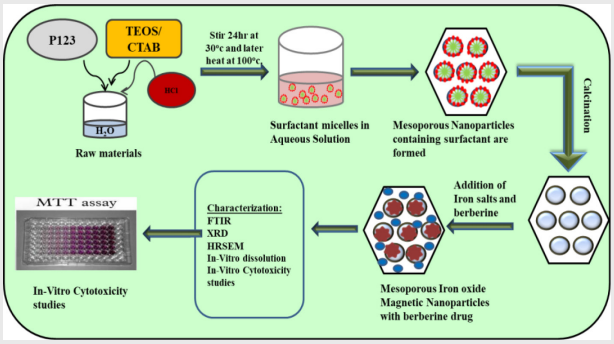
PICTORIAL ABSTRACT



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SUMMARY

In this present study, we formulated four berberine-loaded magnetic nanoparticles using a modified co-precipitation method with calcination. The resulting BBR/MNPs were characterized using FTIR, XRD, HRSEM, zeta potential, VSM, loading efficiency, stability, and in vitro release studies etc. followed by *in-vitro* anticancer studies on MDA- MB-231 cells. The best result was BBR/MCM- 41 MNP with an average particle size 50 nm, which showed good drug loading efficiency and size stability above 7 days. Drug release was maximal (86%) at pH5.5. The MTT- assay confirmed that BBR/MCM-41MNP exhibited more cytotoxicity on MDA-MB-231 cells than BBR, MCM-41MNP. The IC_{50} of BBR/MCM-41 was close that of standard Doxorubicin. BBR/MCM-41MNP showed optimum drug release with potent anticancer activity along with magnetic targeting.

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