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Biocompatibility Studies Of Quercetin Loaded Polymannose Nanoparticles For Potential Chemotherapeutic Application

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Abstract

INTRODUCTION

Quercetin is a natural flavonoid known for its various pharmacological activities, but has poor solubility and bioavailability. Polymannose, a synthetic polymer obtained by the polymerization of mannose, can enhance solubility and is suitable for drug delivery.

AIM

To characterize quercetin loaded polymannose nanosheets (Qur-PM-NS) and investigate its biocompatibility for potential chemotherapeutic application.

MATERIALS AND METHODS:

Qur-PM-NS were prepared and characterized using UV-Vis spectrum, FT-IR and SEM. In vitro drug release study was also carried out to quantify the amount of quercetin resealed in particular time . Biocompatibility was determined by Annexin V PI assay.

RESULTS AND DISCUSSION

The UV-Vis spectrum of Qur-PM-NS gave a strong peak at 381 nm. FT-IR of Qur-PM-NS gave peaks at 3293, 3260, 1649, 1602, 1533, 1451, 1312, 1272, 1160, 1039, 1006, 953, 795, 704, 635, 601 and 571 cm^{-1}. 3293 and 3260 cm^{-1} indicating the presence of various functional groups. SEM analysis gave rod shaped morphology. The average drug release was found to be 3.6 % at 48h. Similarly, in the Annexin V PI assay 78.28% of cells were viable and this is found to be significant.

CONCLUSION

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Qur-PM-NS was found to be biocompatible and can be used for chemotherapeutic applications**.** This is to conclude that we have prepared biocompatible quercetin. loaded Polymannose nanofibres.

Keywords Quercetin, polymannose nanoparticles, therapeutic applications, drug interactions, chemotherapy

INTRODUCTION

Traditionally plants are used for treating various disease conditions (1) (2). The presence of bioactive compounds in plants is responsible for their pharmacological activities (3) (4). Quercetin is a natural flavonoid and a well-known plant phytochemical reported for possessing various pharmacological activities such as anti-cancer, anti-inflammatory, antioxidant, antihypertensive, etc, (5). Quercetin is ubiquitously found in fruits, vegetables, seeds, berries, etc. Even though present naturally, it has poor solubility in water, chemical instability, and relatively low bioavailability (6).

Nutritional quercetin is present mainly as glycosides rather than as aglycones. Quercetin and its derivatives exert key biological effects on cycle progression and cellular signal transduction pathway regulation. Moreover, the metabolic plasticity of quercetin is a determinant in the plant's adaptive reaction. The anti-inflammatory and antioxidant effects of Quercetin are Essential for its activity as an oxidase, and kinase cycle inhibitor, (7). Polymannose is a polysaccharide unit of mannose formed by condensation of mannose which was reported as a suitable drug carrier (8).

In this study Quercetin is encapsulated in fibrous Polymannose Nanosheets (Qur-PM-NS) to enhance its bioavailability, solubility, stability, etc, making it ideal for chemotherapeutic application.

MATERIALS AND METHODS:

Preparation of quercetin-loaded fibrous polymannose nanosheets

Qur-PM-NS was fabricated by adding 2 ml of 40 mg quercetin solution in DMSO into 50 ml of 0.2% aqueous polymannose solution and stirred at 1000 rpm for 30 min. Then 1 ml of 0.5% Tween 80 was added to stabilize the solution. The mixture was then sonicated for 10 minutes. The sonicated solution was centrifuged at 10000 rpm for 20 minutes. The pellet was washed repeatedly with cold water to remove the traces of DMSO through centrifugation. After washing the pellet was resuspended in double distilled water and the suspension was transferred into the petri dish and dried under a freeze dryer. The lyophilized Qur-PM-NS was used for the characterization and bioavailability studies.

Percentage Drug content

About 10 mg of the Qur-PM-NS was dispersed in 20 ml of DMSO and sonicated for 10 mins. Then the solution was centrifuged at 10000 rpm for 30 mins. Quercetin present in the supernatant was using a UV-vis spectrophotometer at 381 nm. The amount of quercetin present in the Qur-PM-NS was calculated using the formula given below

$$\%\ Drug\ Content\ = (\ Amount\ of\ Quercetin/Amount\ of Qur-PM-NS) \times 100$$

Characterization

The Qur-PM-NS were redispersed in water and UV-Vis spectrophotometer of Qur-PM-NS was measured at 300-600 nm. The presence of functional groups was determined by FT-IR (Fourier Transform Infrared Spectroscopy) with the fingerprint region of 3500-1000 cm^{-1}. The structural morphology was analyzed by using Scanning electron microscopy (SEM).

In vitro drug release study

The in vitro release of quercetin from the Qur-PM-NS in PBS (0.1 M, pH 7.4) was estimated using a dialysis membrane of MWCO 3.5 kDa. About 10 mg of the Qur-PM-NS in 10 mL PBS was taken in the dialysis bag and the bag was immersed in a 100 ml beaker with 50 mL PBS. The beaker was incubated at 37ºC in a shaking water bath at 50 rpm. Aliquots of 1 mL were withdrawn from the beaker at different time intervals and replaced with the equivalent amount of fresh PBS. The released quercetin was estimated from the UV absorbance of the sample at 381 nm (9)

Annexin V PI assay

Biocompatibility of synthesized Qur-PM-NS was determined by, annexin V PI assay. Following the approval from the institutional ethical committee, blood was collected from healthy donors. 2 ml of histopaque was added over 2 ml of blood and centrifuged at 2000 rpm for 30 minutes to isolate Peripheral blood mononuclear cells (PBMCs). Isolated PBMCs were cultured in RPMI media containing 10% Fetal Bovine serum (FBS), 1% amino acid L-glutamine, and 1% Penstrep. 100µl of Qur-PM-NS was added to the cultured cells for 12 hrs. Culturing was done in triplicate. For control, cells without Qur-PM-NS were taken. After culturing the cells were stained by adding 5µl of Annexin V and 5µl of propidium iodide and kept at room temperature for 15 mins. Followed by incubation, 400 µl of 1X binding buffer was added to all tubes and observed for apoptosis using BD FACS Lyric flow cytometry. Result analysis was done using FACSuite 4.1 software.

RESULTS

Qur-PM-NS was fabricated using lyophilization. The percentage of quercetin present in nanosheets was found to be 432.92 µg/mg of Qur-PM-NS.

Characterisation studies

The UV-Vis spectrum of Qur-PM-NS gave an intense peak at 381 nm within the wavelength range of 300-600 nm. FIg 1 represents the UV-Vis spectrum of Qur-PM-NS.

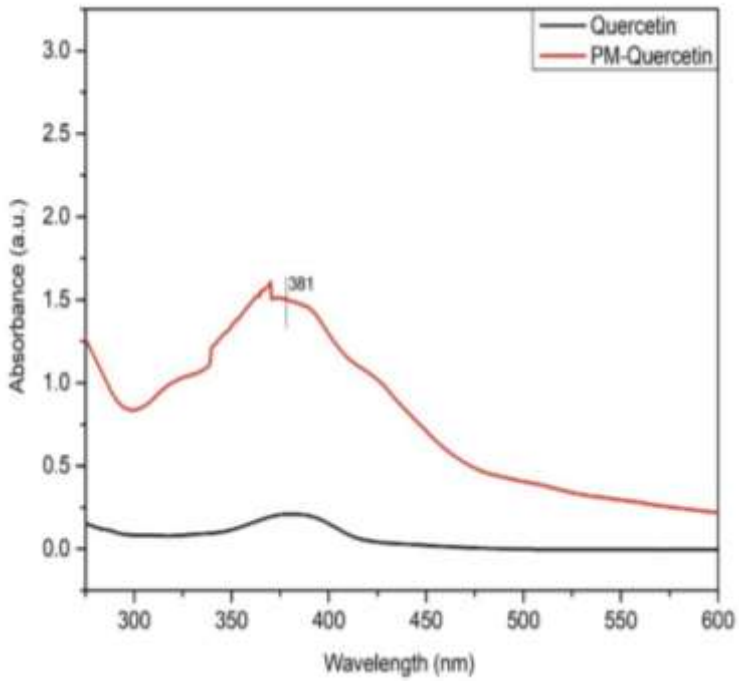

Fig 1. UV-Vis spectrum of Qur-PM-NS**.**

FT-IR gave intense peaks at 3293, 3260, 1649, 1602, 1533, 1451, 1312, 1272, 1160, 1039, 1006, 953, 795, 704, 635, 601 and 571 cm^{-1}. These peaks indicate the presence of different functional groups that act as capping and reducing agents in nanoparticle synthesis. Figure 2 represents the FT-IR spectrum of Qur-PM-NS.

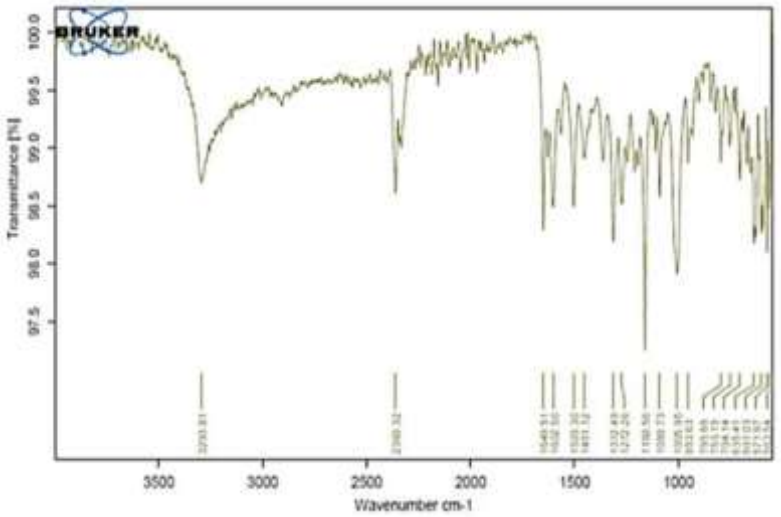

Fig 2 . FT-IR spectrum of Qur-PM-NS

SEM

Structural morphology analysis of Qur loaded fibrous polymannose nanoparticles showed gave rod shaped morphology. Fig 3 represents the SEM images of Qur-PM-NS and quercetin

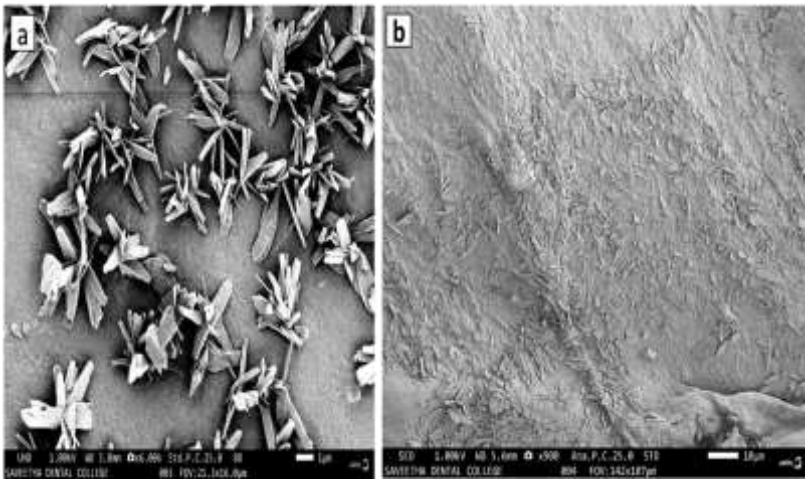

Fig 3. SEM images of Qur (a) and Qur-PM-NS (b).

Drug Release Study

Percentage of Quercetin released from polymannose at pH 7.4 is represented in Fig 4. The amount of quercetin released is found to be 3.6 % at 48 h..

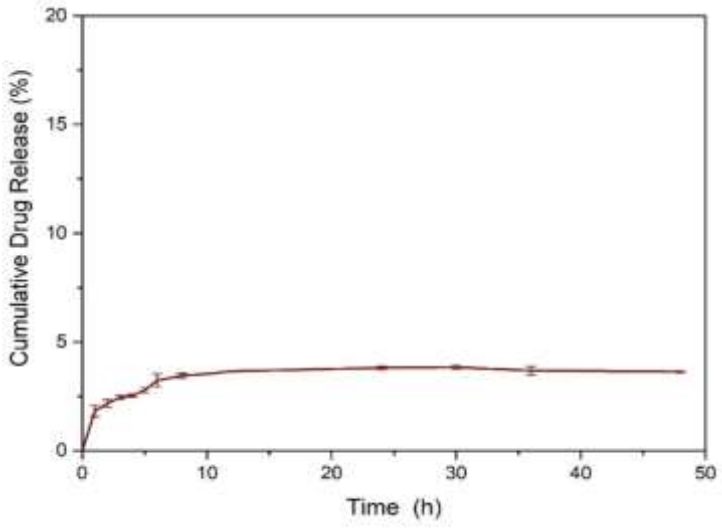

Fig 4 Cumulative quercetin release from Polymannose

Annexin V PI assay

The result for annexin V PI assay was interpreted in four quadrants. The Lower left quadrant (LL) represents the percentage of viable cells post-treatment withQur-PM-NS, The Lower right quadrant represents (LR) the percentage of cells in early apoptosis, while the upper right quadrant (UR) represents the percentage of cells in late apoptosis and Upper left quadrant (UL) represents the percentage of cells in necrosis. From the annexin V PI assay, the percentage of viable cells post-Qur-PM-NS treatment was found to be 78.28%, while 21,60% of cells were in early apoptosis, 0.03% of cells were in late apoptosis and 0.10% of cells were found to be in necrosis stage. The results were found to be concordant with the control. Fig 5 represents the annexin V PI assay of control and Qur-PM-NS.

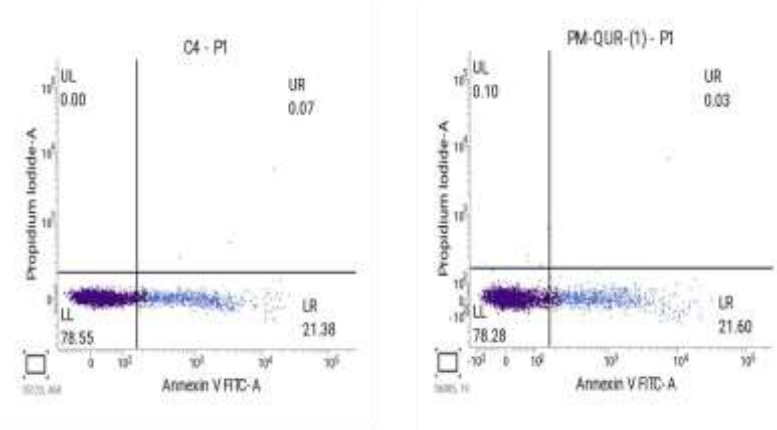

Fig 5 Annexin V PI assay of Qur-PM-NS

Biocompatibility and drug release studies, blood sample was collected from healthy volunteers after getting approval from the institution's ethical approval board. We took a large beaker and added 2 ml of 20 mg of quercetin in DMSO and stirred for 10 min at 1000 revolutions per minute. Then added 500 µL of 0.5% Tween 80 and centrifuged at 6000 revolutions per minute then died the pellet using a lyophilizer.

DISCUSSION AND CONCLUSION

The use of nanotechnology in the field of medicine and biomedical research has attained tremendous growth this past decade (10) (11). It is extensively studied in the field of cancer therapeutics to improve pharmacokinetics and reduce the toxicity of chemotherapeutic drugs. Recently researchers are focusing on nanocarriers to deliver chemotherapeutic drugs directly at the site of the cancer cells with minimum drug elimination (12) (13). In 2021, Liang et al. synthesized a nanomaterial made out of both organic and inorganic material coated with a polymeric material made out of hyaluronic acid, which can accumulate in liver cancer and release drug (14). Another study demonstrated the transfer of photophysical and photochemical properties obtained by using nanostructured phthalocyanine (15). Considering this, we have coated quercetin onto fibrous polymannos nanosheets. We have used quercetin impregnated onto fibrous polymannos nanoparticles., and characterized their properties using techniques such as UV-Visible Spectral analysis, FT-IR, and SEM, along with a drug release study and toxicity study was evaluated on PBMCs using Annexin V-PI apoptosis assay. The quercetin loading is confirmed by the FT-IR spectrum. FT-IR spectrum results showed strong peaks stretching at 3293, 3260, 1649, 1602, 1533, 1451, 1312, 1272, 1160, 1039, 1006, 953, 795, 704, 635, 601, and 571 cm^{-1}. 3293 and 3260 cm^{-1} peaks correspond to O-H stretching, indicating the alcohol group. 1649 cm^{-1} corresponds to C=C stretching indicating the alkane group. 1602 cm^{-1} corresponds to N-H bending indicating an amine group. 1533 cm^{-1} corresponds to N-O stretching indicating nitro compound. 1451 cm^{-1} corresponds to C-h bending indicating an alkane group. 1312 cm^{-1} corresponds to O-H bending indicating the phenol group. 1272 cm^{-1} corresponds to C-O stretching indicating aromatic esters/amine. 1160 cm^{-1} corresponds to C-O stretching indicating aliphatic ether. 1039 cm^{-1} corresponds to S=O stretching indicating sulfoxide. 1006, 953, 795 and 704 cm^{-1} correspond to C=C bending indicating alkene. 635, 601 and 571 cm^{-1} correspond to C-Br stretching indicating halo compounds. The SEM results of Quercetin loaded onto the fibrous polymannose nanoparticles showed rod-shaped quercetin distributed in the polymannose nanosheets. In our drug release study, the average drug content was found to be 432.92. Apoptosis assay revealed that almost 78.28 % of cells were alive after treatment with 100 µg of Qur-

PM-NS, comparable with that of untreated control showing 78.55 % viable cells; this confirmed the biocompatibility of Qur-PM-NS and their non-toxic nature. Therefore, our *in-vitro* study showed that Qur-PM-NS did not induce any significant apoptosis. Therefore, we concluded that the quercetin-impregnated fibrous polymannose nanoparticles are economic. Because of its biocompatibility, its potential role as an efficient nanocarrier to release chemotherapeutic drugs is investigated in-vitro in this study. However, an extensive investigation must be carried out to evaluate the *in-vivo* toxicity nature of the Qur-PM-NS and to deliver chemotherapeutic drugs directly at the site of the cancer cell.

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