Preparation of curcumin-loaded mesoporous silica and its evaluation of ex vivo and antioxidant profile to suggest further study

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Abstract:

In this article, we constructed a very novel carrier in the form of nanoparticles, which is a core-shell structure, and applied it for the delivery of curcumin to study the toxicity and dose profile of the drug. In addition, the use of this carrier, i.e. silica nanoparticles, results in a controlled release behaviour of curcumin. The nanocomposite structure was prepared by the simple process of mesoporous silica nanoparticles seen to have higher surface area. It also provided a large accessible volume for the good absorption of the drug.

Keywords:

Curcumin, Silica Nanoparticle, Dose Profile, Mesoporous Silica, Nanoparticle

Background:

According to the WHO estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030 [1]. According to International diabetes federation, India had 50.8 million diabetic adults in the year 2010 and this would increase to 87 million by the year 2030. The prevalence of diabetes is five times higher in urban population than in rural area, due to urbanization and high in southern region as compared to northern and eastern region of the country [2]. Health care economics of diabetes is less explored discipline in India. The lack of access to health care services, national welfare schemes and health insurance coverage for diabetes makes the treatment unaffordable resulting in late diagnosis and increased cost in treatment of diabetes and early onset of complications. As per the current diabetic estimate of 50.8 million diabetics in India, the recent study states that the expenditure towards direct and indirect cost incurred would be $31.9 billion while the allotted national health budget for the fiscal year 2009-2010 was a meagre of $4.5 billion.

Material and Methods:

Material:

Cetyltrimethylammonium bromide (CTAB) (ottochemica purity ≥ 99%), Tetraethylorthosilicate (TEOS) (Alfaaesar chemicals limited purity≥ 98%), Ethanol (Mercpurity ≥99%), Dionised water, Sodiumhydroxide, Concentrated Hydrochloric acid, Polaxamer, Sodiumdihydrogen Orthophosphate dehydrate (Fisher scientific Purity ≥98), Acetonitrile HPLC grade, Methanol (CDH purity 99.5%), Curcuminoid (Gift sample Karnataka antibiotic)

Method:

Preparation method of Silica nanoparticles:

The different amount of the silica nanoparticle was prepared by slight change in the process as reported (Hom et al., 2010**)** by taking different concentration of CTAB (2.8 gm), 2.0 M Sodium hydroxide (3.9 mL), and water (100mL) putted it at 80^0C for 30 min. when fully clear solution is observed then TEOS 3.3 g is rapidly added with help of injection and rapid stirring nearly 600 rpm after continuous stirring for four minutes there is observance of white precipitate the temperature maintained at 80^oC for 2.5 hours. Then the product was diluted three times with distilled water nearly (300mL) and filtered simultaneously. Then it is washed with methanol and water solution in ratio (2:5) two times further its acid extraction was done with the methanol (100mL) conc. Hydrochloric acid (1mL) mixture and previously prepared sample nearly 3.3 g at 60^oC for 6.5 h using hot plate. The resulted sample was then washed with water and methanol several time until all the surfactant (CTAB) were removed and then the solid product was collected by the centrifugation at 2000 rpm (CPR-30 Plus, REMI, India). The process was done with different concentration of the above used chemicals to

obtain different types of the mesoporous silica nanoparticles MCM-NPs and the given table shows the concentration of the chemicals and name of sample obtained.

***In vitro* release study through High performance liquid Chromatography analysis:**

Liquid chromatograph Shimadzu LC-2010 CHT (M/s Schimadzu Co. Ltd., Chiyoda ku, Tokyo, Japan) equipped with 4.6 mm × 250 mm Merck HPLC column RP-18, ODS with particle size 5 micron and PDA detector with 284 nm wavelengths was used for the determination of in vitro release study. About the 5 mg of the sample MCM-NPs-C-CAR and 5 mg pure CAR was suspended in the 2mL of the 1% sodium lauryl sulphate (SLS) with phosphate buffer saline having pH 7.4 with cellulose membrane of fixed cut off (MW cut-off 5000, Hi-Media) separately. The dialysis bags were placed in 50 mL of the phosphate buffer saline(7.4pH) solution (sink condition) with magnetic stirring at 75rpm and then 1.5 mL aliquots were extracted at different time intervals and replaced with fresh buffer solution of same amount 1.5mL and after 24hrs the sample was analyzed with reverse phase HPLC C_{18} column [3-4]. The used solvent was carbinol of HPLC grade with water of HPLC grade and acetonitrile of HPLC grade (50:50:80 v/v) and before using it was filtered with membrane filter of 0.22µm and flow rate was maintained 1mL/min the experiment was revised double time for the analysis of variability obtained with each time. The obtained chromatogram was analyzed further.

Ex vivo study:

Percentage live red blood cells (RBC) count study:

As per suggested protocol percentage live RBC was calculated by subtracting the percent haemolysis from the total haemolysed sample i.e., haemolysis by distilled water the percentage of live blood cells was calculated of samples naïve drug CAR, blank MCM-NPs-

C and final loaded drug MCM-NPs-C-CAR. Human blood sample was calculated from the healthy human 8 mL within the EDTA storage vial and then the blood sample was centrifuged at 2500 rpm (R-4C DX, REMI, India) and then the RBC was collected and instantly suspended into normal saline solution (0.9%w/v). Then sample which should be analyzed prepared of (20ppm) and placed 4mL each sample and then equal amount of RBC are placed to each samples and let it for the incubation period of the 30 minutes and after incubation it was centrifuged and supernatant was analyzed as it is by ultraviolet visible spectrophotometer (Cary series-100, UV-visible spectrophotometer, AgilentTech.) [5,6,7]. RBCs in distilled water considered as 100 percent haemolysis or no live RBCs left and the absorbance of distilled water is taken as a reference for other samples

% RBC Live = % haemolysis DW - %haemolysis of Samples

For % Haemolysis = $[a_s \div a_{100}] \times 100$

Where,

a_s=absorbance of sample

a_{100}= absorbance of distilled water

In the above report time dependent percent RBCs live are counted i.e., 30 minutes, 12hoursand 24hrs respectively.

Protein binding study:

A Solution of BSA was prepared (2% w/w) of PBS saline of pH 7.4 for the study. CAR (1mg) and MCM-NPs-C-CAR (equivalent to 1 mg drug) were added to 1 mL of BSA solution and packed into dialysis bags, separately. These dialysis bags were dipped into 20 mL of PBS of pH 7.4 under stirring at 37±1 ℃. Samples were analyzed

spectrophotometrically which were taken at the intervals of 1 h, 2 h, 3 h, 4 h, 5 h and 6 h. The calculations of percent protein drug binding were performed, as per the equation [8-9].

$$\%\ Protein\ drug\ binding = \frac{(Theoritical\ amount\ of\ drug\ in\ bag - Drug\ in\ sink)}{Theoritical\ drug\ in\ bag} \times 100$$

***In-Vitro* Antioxidant Study by DPPH Method:**

DPPH free radical scavenging assay were used for determining antioxidant activity of HAF/HLO as mentioned by Nithianitham et al and Zuraini et al with some modifications. 10mg/mL stock solution of HAF/HLO was prepared. Different dilution of HAF /HLO (20 µLto 100 µL) was taken and was diluted up to 1 mL with methanol. Then 1mL of each dilution was added with 2 mL of 0.004% (w/v) DPPH solution. This mixture was vortexed, kept inside the incubator for 30 minutes in dark, and spectrophotometric absorbance was measured at 425 nm. 80% (v/v) methanol was used as blank solution. Ascorbic acid was used as the standard compound for comparative study. All measurements were done in triplicate. Following formula was used to calculate DPPH free radical scavenging activity:

Scavenging activity (%) =

Here, control =0.004 % (w/v) DPPH solution; sample = HAF/HLO

The result was reported as IC50 value and ascorbic acid equivalents (AAE, mg/g) of HAF/HLO

***In-vitro*-release study by HPLC analysis:**

It is observed that the almost 95 percent of the drug release in 6hrs and the MCM-NPs-C-CAR favours sustained release profile and it is seen that almost 75 percent of the drug released in the media take almost 12hrs and after 20hrs the release pattern was seen constant.

The *in vitro* drug release is explained (Fig-1) by the first order kinetics and (r^2=0.9132) showing the good class of release of the drug.

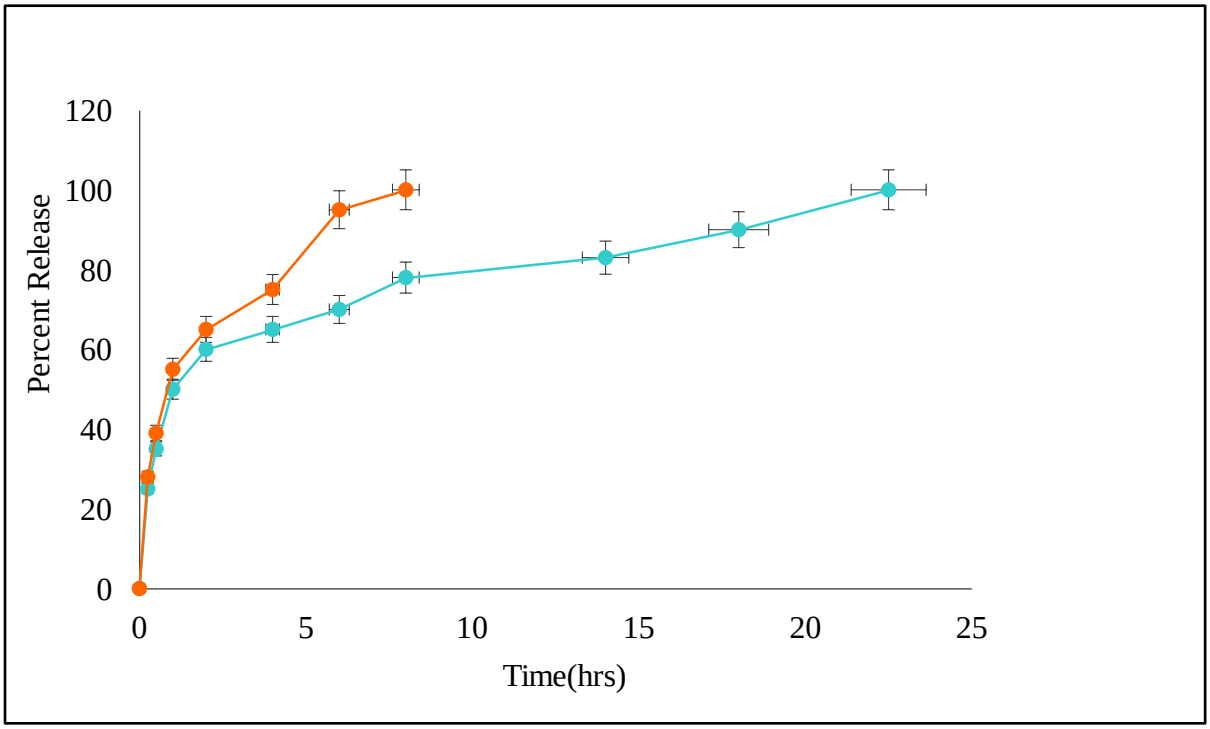

Figure 1 Release Profile of CAR and MCM-NPs-C-CAR

***Ex-vivo* study:**

Percentage live RBCs count:

The live RBCs are counted in percent for the confirmation of the formulation that what is the haemolytic percentage and result of distilled water, normal saline, CAR, MCM-NPs-C and the final formulation MCM-NPs-C-CAR is giving the result 0%,99.14%,98.63%,99.66%and 98.8% respectively (Fig-2). Distilled water is taken as the reference sample with no live RBCs and all the formulations are dissolved in the normal saline and volume was also make

up with the same saline. Therefore, the above stated result support the good behaviour in reference to the haemolytic toxicity. The result also suggest that the silica Nano-formulations are very fortunate means for the targeted drug delivery.

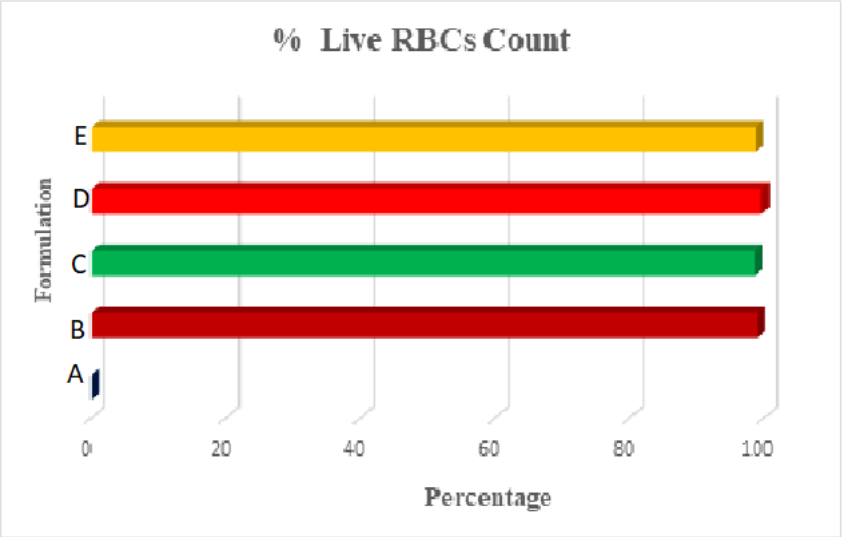

Figure 2 Percentage of Live RBCs Count

Protein binding study:

The biding efficiency of the pure drug CAR and the drug loaded silica nanoparticle MCM-NP-C-CAR at time interval of 0 to 6 hrs and it is observed to be (74.54%) in case of the pure drug and (62.31%) observed in the drug loaded nanoparticle (Fig-3). It is confirming with this data that the protein binding is lesser in drug loaded silica nanoparticles which reveals that the prepared formulation has very good penetration power to the cell. Therefore, the significant difference is observed between the CAR and MCM-NP-C-CAR.

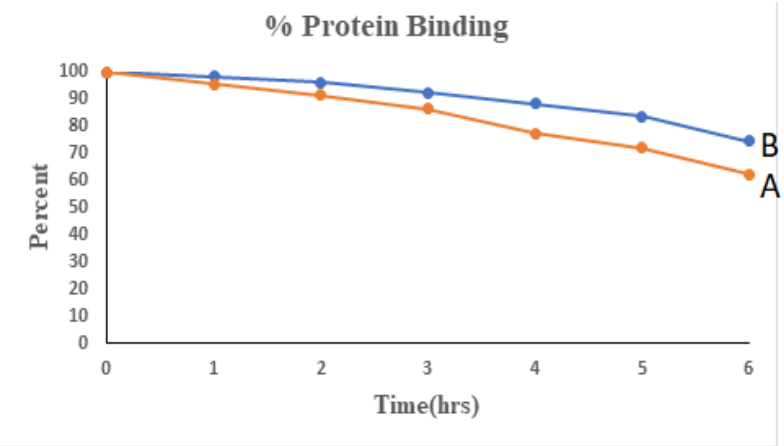

Figure 3 Percentage of Protein Binding

Antioxidants have the ability to scavenge free radicals in the human body and have been suggested to contribute to the protective effect of plant-based foods on diseases such as cardiovascular disease, cancer, and type 2 diabetes. However, evidence from supplementation studies using various antioxidants, including vitamin C, vitamin E, carotenoids, zinc, or selenium, does not support the hypothesis that antioxidants decrease risk of these diseases. Intervention studies highlight a lack of information on the safety of sustained intakes of moderate to high doses of micronutrient supplements and suggest that long-term harm cannot be ruled out, particularly in smokers. The observed values of HAF's scavenging activity at different concentrations were depicted as the plotted graph. IC50value of HAF and ascorbic acid were calculated as 114.24 µg/mL and 26.86 µg/mL respectively.

Values represent the mean ± SEM (n = 3);

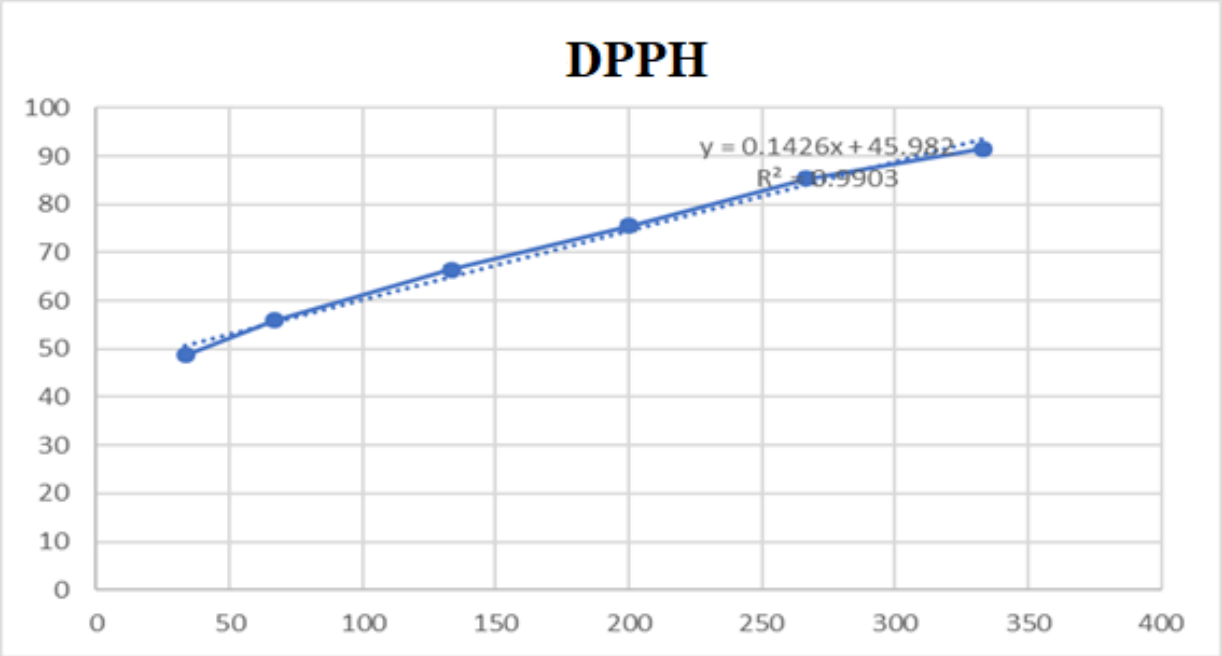

Figure 4 DPPH Assay

Conclusions:

The proposed plan concludes that the prepared formulation with nano drug delivery with curcumin shows high efficiency to combat with the diseases and it is also intervein with all the activities the invitro release of the nanoparticles shows the sustained release pattern and percent hemolysis is very low so it is very good to administer the formulation and patient compliance. There is also the lower protein binding platform and their antioxidant profile suggestive to the formulation for recommendation and they are highly suggestive to the patient and further recommended to animal study model.

Abbreviations:

MCM-NPS-C	Mesoporous Silica Nanoparticles
MCM-NPs-C-CUR	Curcumin loaded Mesoporous Silica Nanoparticles
UV	Ultra violet
FTIR	Fourier transform infrared analysis
SEM	scanning electron microscopy

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