10

Cell cytotoxicity study

10.1 Significance

After the synthesis and successful loading of MSNs with anticancer drugs, it was necessary to evaluate toxicity of developed MSNs. The possible cytotoxicities of nano-materials could result from cellular injuries through a variety of mechanisms, such as membrane peroxidation, glutathione depletion, mitochondrial dysfunction, and DNA damage, eventually leading to cell death[1]. Hence, systematic examinations concerning the biocompatibility of nano-materials are necessary prior to their medicinal use.

MCM-41 and MSU-H MSNs have distinguishable differences in their individual lattice spacing, pore diameter, wall thickness, surface area, and shape regularity[2-4]. The cell cytotoxicity of MSNs depends on several factors like mesoporosity, functional group, extend of endocytosis, incubation time, particle dosage, particle endocytic efficiency, particle chemical properties, particle physical properties and above all the type of cell culture used for the study. Literature survey showed some studies of MCM-41 and MSU-H for their cytotoxicity behavior by in-vitro cell cytotoxic studies[5-16].

10.2 Introduction

The objective of the present study was to improve the solubility of two poorly soluble anticancer drugs using MCM-41 and MSU-H MSNs carrier so the cell cytotoxicity study of drug loaded MSNs was carried out with two different cell lines. One of them is cancerous cell line and other is normal or non-cancerous cell line. The selected cell lines were Human myeloid leukemic cells, K-562 (cancerous cell line) and human lung alveolar epithelial cells, L-132 cell lines (normal or non-cancerous cell line). Rational behind the selection of K-562 cell line is that it is a cancerous cell line and the study was conducted for the anticancer drugs that are therapeutically useful for human myeloid leukemia[17-22] whereas L-132 cells were selected for their relevance and wide utility in the development of drug and gene delivery vehicle[23-29].

There are many cytotoxicity assay techniques available, like clonogenic/non clonogenic[30], H-thymidine uptake test[31], colorimetric assay[32] (commonly MTT assay and trypan blue technique), LDH leakage assay[33], caspase activity[34], luminescence assay[35] etc. Choosing one of them was a very challenging task. The cell viability assay is chosen on the basis of sensitivity of detection, ease of use, reproducibility of data, total running cost, availability of instrumentation and reagents stability at ambient temperature.

For the present study MTT (3-[4-5-dimethylthiazol-2-4]-2, 5-diphenyltetrazolium bromide) assay technique was used. In MTT assay tetrazolium salts have been used as indicators of both biological redox systems and viability of the cells[32]. They are a group of heterocyclic organic compounds that form highly colored and insoluble

formazan after reduction. In particular, the molecular cation (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium) is a very important redox indicator and used in pharmacology and related research areas. This assay technique is comparatively simple, sensitive and reproducible with a low running cost.

10.3 Principle of the study

MTT assay is the most common assay methods used by researchers to measure the cell viability[36,37]. MTT assay technique is a quantitative method and more sensitive. A linear relationship between cell viability and absorbance can measure the growth rate and death rate.

MTT assay method was used to measure cell viability. MTT is a non radioactive cell proliferative assay which measures the reduction of MTT by the mitochondria and/or cytoplasmic succinate dehydrogenase enzyme and to form an insoluble, dark blue insoluble formazan product[38]. Only viable cells having dehydrogenase activities are able to reduce significant amounts of MTT dye to formazan. The amount of color produced is directly proportional to the number of viable cells. Most of the mammalian, plant, and yeast cell can be used for the cell viability study during MTT assay.

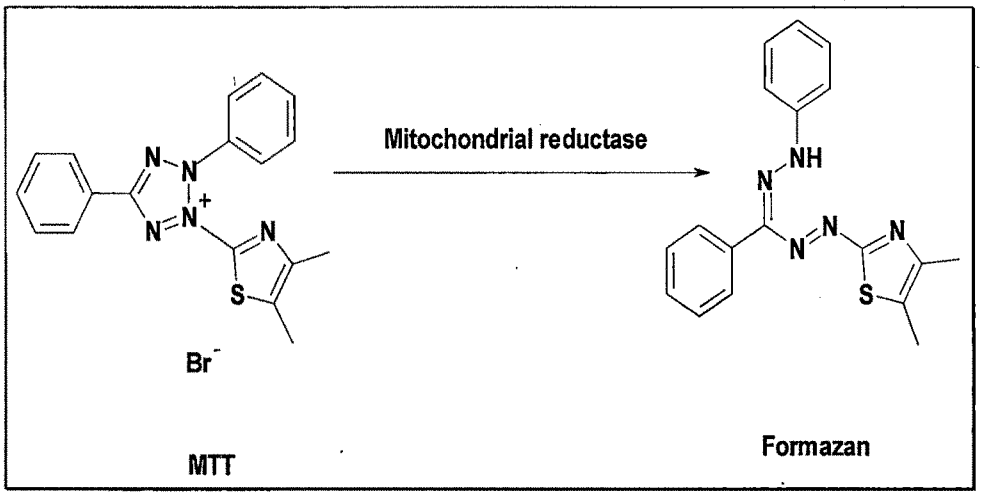


Figure 10.1: MTT assay principle

The prime objective of this experiment was to study the effect of surface chemistry and morphology of MSNs as a function of concentration and incubation time on viability of cancerous and (K-562) non-cancerous (L-132) cells by MTT assay. The selected cells were treated with drugs, their formulations and placebo (MSNs without drugs). The experimental results will provide ideas about possible cytotoxic effect of MSNs.

Materials and Methods

10.4 Materials and methods

10.4.1 Cell lines

The human chronic myeloid leukemia (CML) K-562 cell line and the human lung alveolar epithelial cells L-132 were purchased from National Centre for Cell Science (NCCS, Pune, India). Fetal bovine serum (10% v/v), L-glutamine, Sodium bicarbonate (NaHCO3), phosphate buffer saline and MTT were purchased from Himedia, (Mumbai, India). Antibiotics (Penicillin/streptomycin) were obtained from Sigma Aldrich (Mumbai, India). Trypsine (0.25%) was obtained from Hyclone (Fisher Scientific). 96 well plates (flat bottom, Polystyrene) and cell culture flasks (175 cm^2 polystyrene) were purchased from Tarson (Mumbai, India). All other chemicals used were of research grade.

10.4.2 Preparation of stock solutions

Stock suspensions of the drug loaded MSNs (MCM-41 and MSU-H) were prepared in dimethyl sulfoxide (DMSO) and diluted by nutrient medium to various working concentrations, and used immediately. The nutrient medium was RPMI 1640 medium, supplemented with l-glutamine (3 mm), streptomycin (100 mg/mL), penicillin (100 IU/mL), 10% fetal bovine serum (FBS), and 25 mm HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) and adjusted to pH 7.2 with bicarbonate solution. MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was dissolved (5 mg/mL) in phosphate buffered saline pH 7.2 and filtered through a millipore filter (0.22 mm) before use.

10.4.3 Cytotoxicity of drug loaded MSNs by MTT assay

Cytotoxicities of MSNs were evaluated on K-562 and L-132 cells by using the standard cell counting kit. A 96-well plate was utilized for the cell placement. 100 µl/well cell-free media or cell suspension was distributed into a row of at least 6 wells. For K-562 cells, 4000 cells per well and for L-132 cells, 6000 cells per well were plated 24h before the addition of MSNs. Cells were immediately treated with MSNs of different concentrations (100, 200, 400, 800, and 1000 µg/ml, respectively). Plates were then incubated at 37 °C with 5% CO_2 for different time duration (3, 17, 51, and 96h).

10 µl of MTT solution (5 mg/ml in phosphate buffered saline) was gently added to each well up-to 96h. Samples were incubated for a further 4h at 37 °C in a humidified atmosphere with 5% CO_2. Then, 10% SDS (100 mL) was added to the wells. The absorbance was measured at 550 nm the next day. Cell survival percentages were determined by the absorbance at 550 nm of a sample of cells grown in the presence of various concentrations of agent divided by the absorbance of a control sample (the absorbance of cells grown only in nutrient medium), after subtracting the absorbance of the blank from the absorbance of the corresponding sample with target cells.

Results and Discussion

10.5 Results and discussion

In-vitro cytotoxicity investigation can provide some preliminary knowledge to understand the advantages of the drug loaded MSNs (formulation) over the free drug. In order to determine the effect of the MSNs on the cells as well as the sensitivity of selected cells (K-562 and L-132 cells) to the selected drugs (MTX and DTB), the cytotoxic effect of MSNs was evaluated by its inhibitory effect on the cell viability.

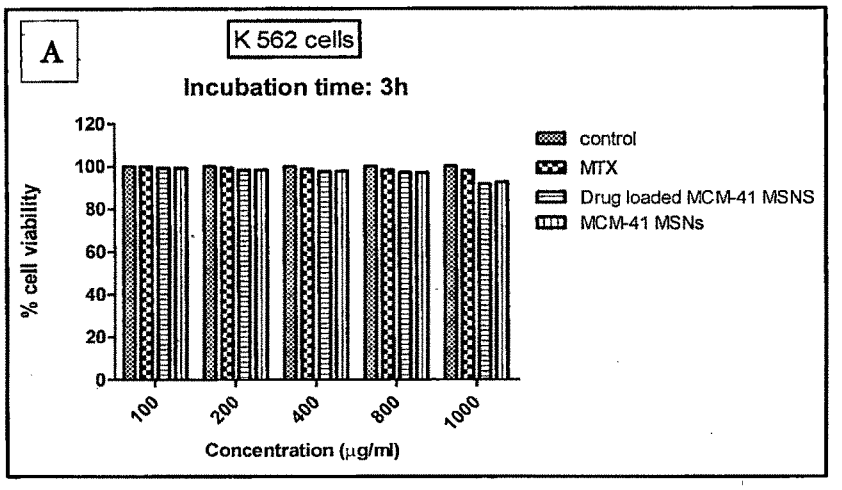
The cytotoxic activity and subsequent therapeutic potential of drug loaded MSNs (formulation) were evaluated by incubating selected cells within increasing range of concentrations from 100 to 1000 over four different incubation period i.e. 3, 17, 51 and 96h. In the present *in-vitro* study the cytotoxicity of different formulations (MTX loaded MCM-41 & MSU-H MSNs and DTB loaded MCM-41 & MSU-H MSNs) was evaluated on two different cell line (K-562 and L-132) using MTT assay. It is assumed that media supports cell growth fully and hence the cell viability with media treatment group (control) is considered 100%.

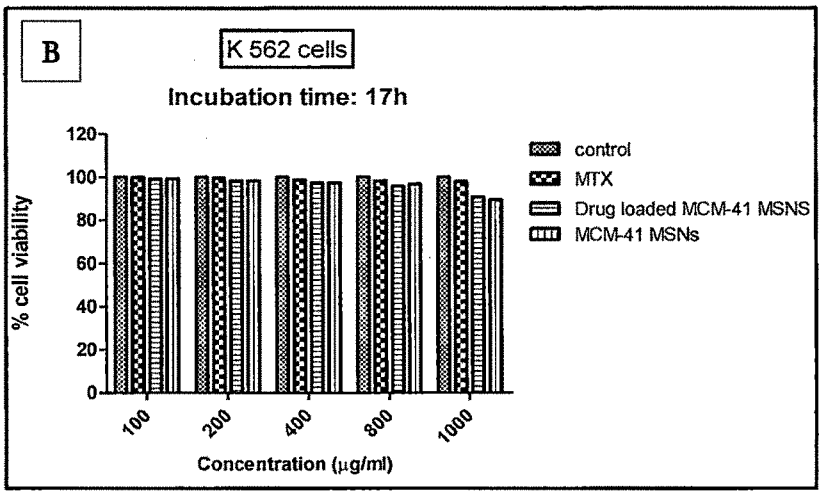
10.5.1 MTX loaded MSNs (MCM-41 and MSU-H) and K-562 cell line

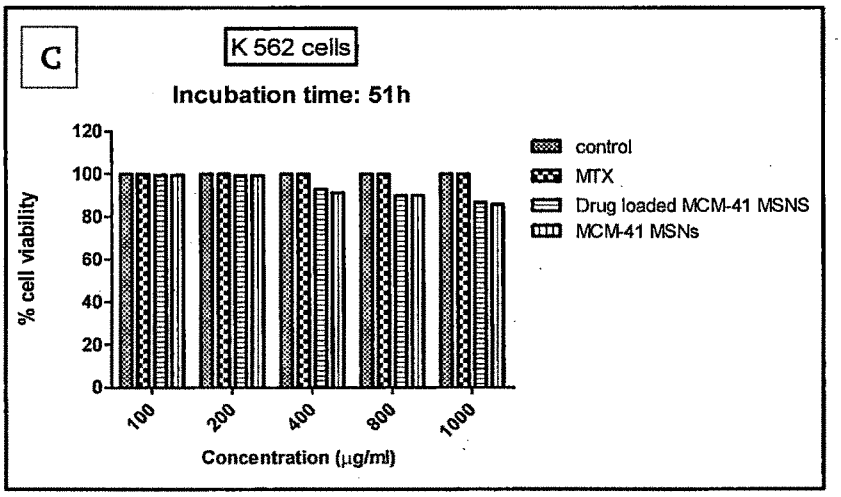
The cellular viability of K-562 cells was investigated over various concentrations of MTX and their MCM-41 formulation and/or placebo (MCM-41 MSNs). Fig. 10.2 shows the cell viability at different durations of incubation time (3, 17, 51 and 96h). For all tested incubation times, the cell viability was found to be more than 80%. The cell viability data for MTX loaded MCM-41 and its placebo after incubation of 96h is reported in Table10.1, which shows that MCM-41 MSNs alone and/or drug loaded MCM-41 MSNs were devoid of any significant cytotoxic effect on K-562 cells. It was observed that at concentrations up to 400 µg/ml more than 90% of the cells are viable. Nevertheless, in presence of high concentration (1000 µg/ml) of MTX loaded MCM-41 and its placebo, the cell viability was more than 80%.

Table 10.1: Percentage viability of K-562 cells against MTX + MCM-41 MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	MTX	MTX loaded MCM-41 MSNs	MCM-41 MSNs
100	99.89	98.74	98.47
200	99.75	98.24	98.30
400	99.42	91.89	90.71
800	98.97	86.65	86.87
1000	98.80	81.25	80.99


A
K 562 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MCM-41 MSNS
MCM-41 MSNs


B
K 562 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MCM-41 MSNS
MCM-41 MSNs


C
K 562 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MCM-41 MSNS
MCM-41 MSNs

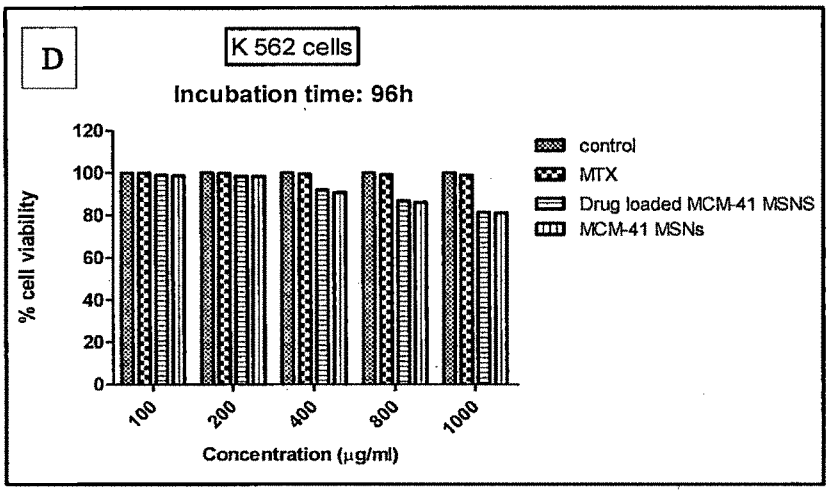
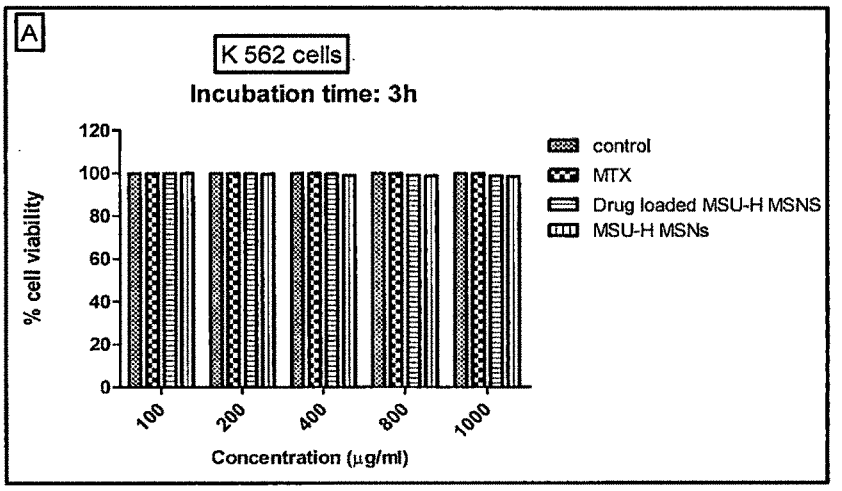


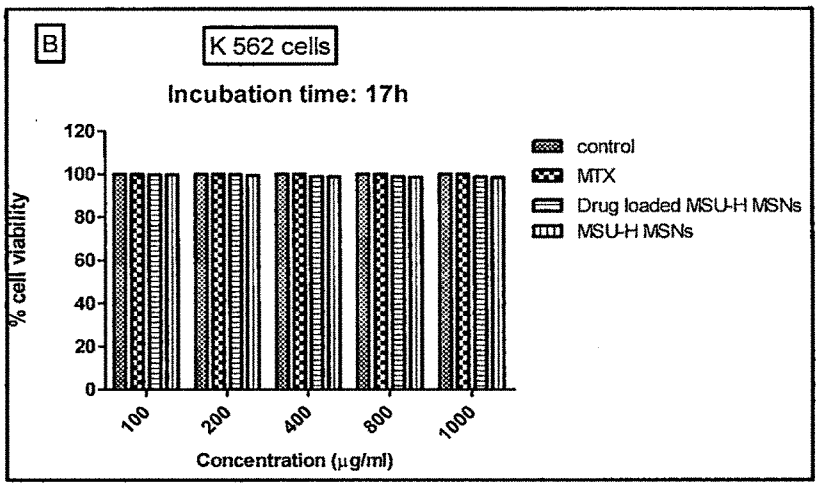
Figure 10.2: Effect of MTX + MCM-41 MSNs on cell viability of K-562 cells at different concentrations and incubation time (A-D)

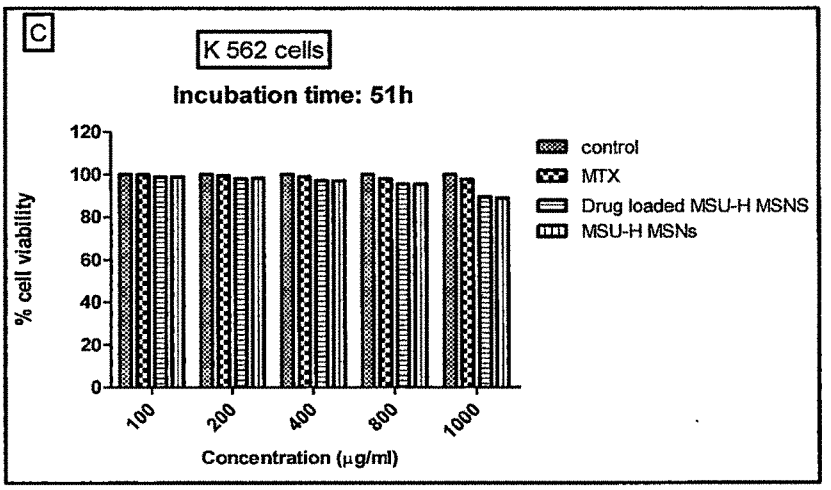
Similar findings were observed for the MTX loaded MSU-H MSNs. The result of cell viability study showed that MTX, MTX loaded MSU-H MSNs and MSU-H MSNs are significantly not toxic to K-562 cells. The cell viability was checked at different incubation times (Fig. 10.3). The results obtained at each incubation time revealed that the viable cells are more than 85%. Percentage cell viability after the incubation period of 96h is shown in Table 10.2. It was observed that at concentrations up to 400 µg/ml more than 96% of the cells were viable and in presence of high concentration (1000 µg/ml) of MTX loaded MSU-H and its placebo, the cell viability was more than 85%.

Table 10.2: Percentage viability of K-562 cells against MTX + MSU-H MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	MTX	MTX loaded MSU-H MSNs	MSU-H MSNs
100	99.78	98.49	98.42
200	99.31	97.94	97.35
400	98.58	96.83	96.53
800	97.98	94.98	95.43
1000	97.82	86.81	85.50


A
K 562 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MSU-H MSNS
MSU-H MSNs


B
K 562 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MSU-H MSNs
MSU-H MSNs


C
K 562 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MSU-H MSNS
MSU-H MSNs

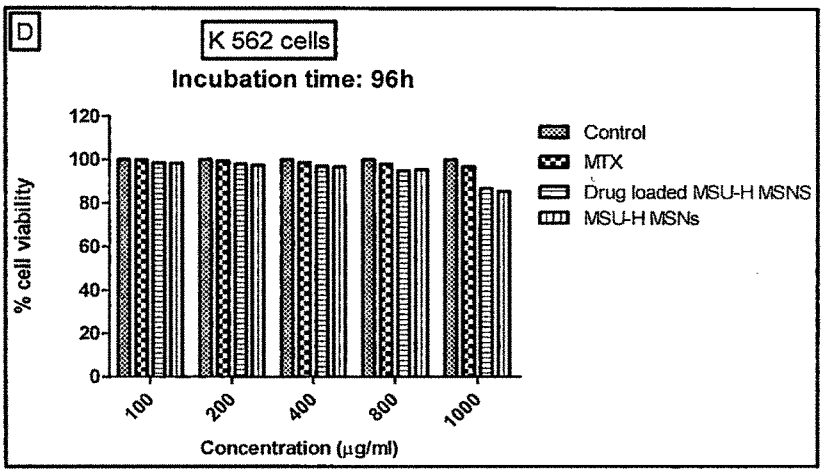


Figure 10.3: Effect of MTX + MSU-H MSNs on cell viability of K-562 cells at different concentrations and incubation time (A-D)

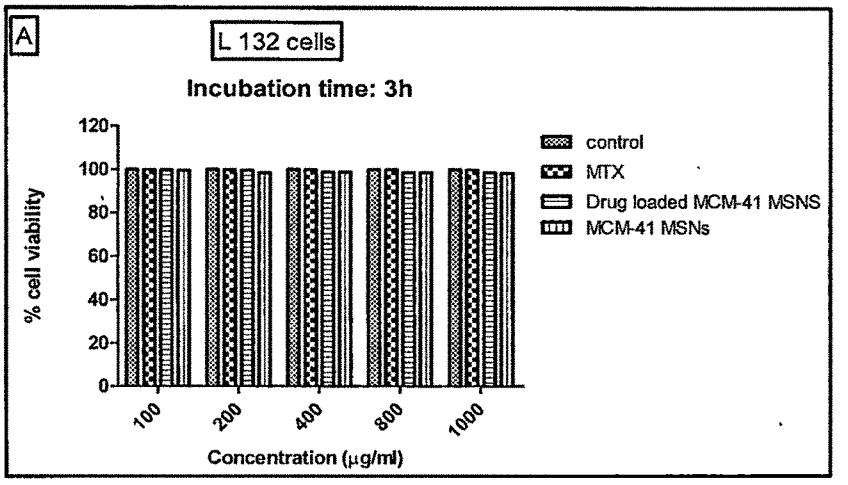
10.5.2 MTX loaded MSNs (MCM-41 and MSU-H) and L-132 cell line

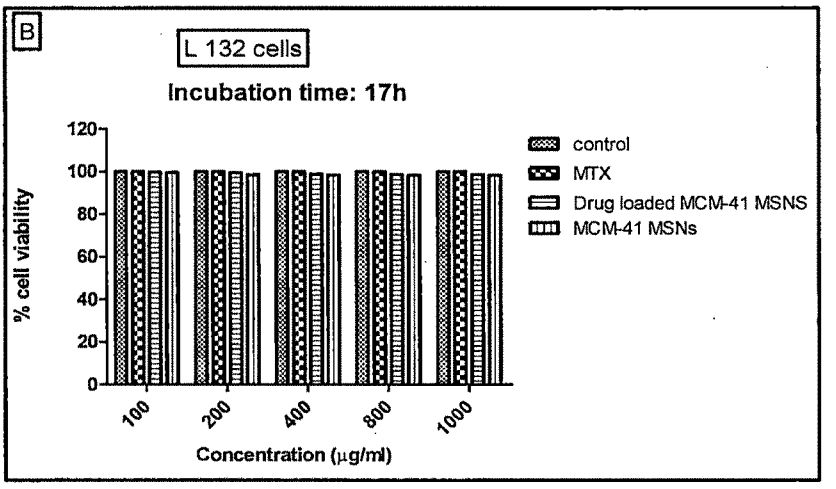
The cell cytotoxicity study was carried out on non-cancerous cell line, L-132 cells. The study was performed in similar manner as with cancerous cells and MSNs were checked for cytotoxic behavior on normal cells.

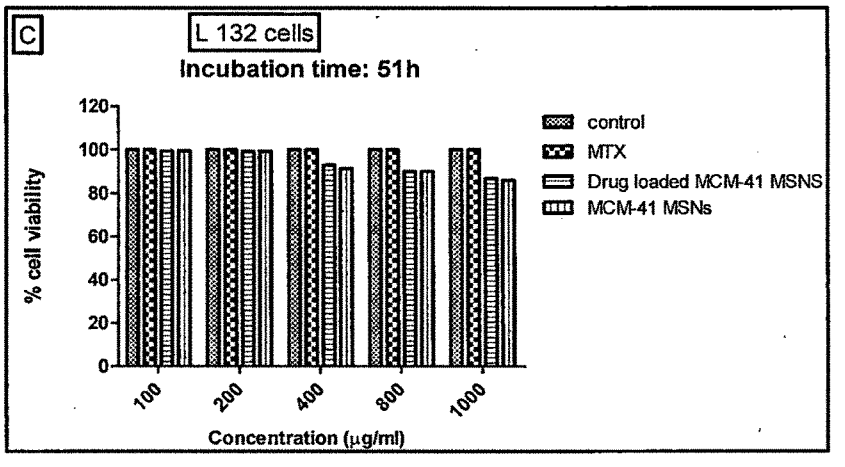
The cell viability of L-132 cells were investigated over various concentrations and incubation times for MTX, MTX loaded MCM-41 MSNs and MCM-41 MSNs. The cell viability of L-132 cells at different concentrations and incubation times is shown graphically in Fig. 10.4. The cytotoxicity assay result revealed that more than 80% cells are viable after 96h of incubation. Table 10.3 reports the data of cell viability after 96h of incubation of MTX loaded MCM-41 and its placebo, clearly revealing that MCM-41 MSNs alone and drug loaded MCM-41 MSNs were devoid of any significant cytotoxic effect. It was observed that at concentrations up to 400 µg/ml more than 90% of the cells were viable, whereas in presence of high concentration (1000 µg/ml) of MTX loaded MCM-41 and its placebo, the cell viability was found to be more than 82%.

Table 10.3: Percentage viability of L-132 cells against MTX + MCM-41 MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	MTX	MTX loaded MCM-41 MSNs	MCM-41 MSNs
100	99.89	98.74	98.47
200	99.81	98.24	98.30
400	99.74	91.89	90.71
800	99.69	86.65	85.87
1000	99.65	82.25	82.99


A
L 132 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MCM-41 MSNS
MCM-41 MSNs


B
L 132 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MCM-41 MSNS
MCM-41 MSNs


C
L 132 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MCM-41 MSNS
MCM-41 MSNs

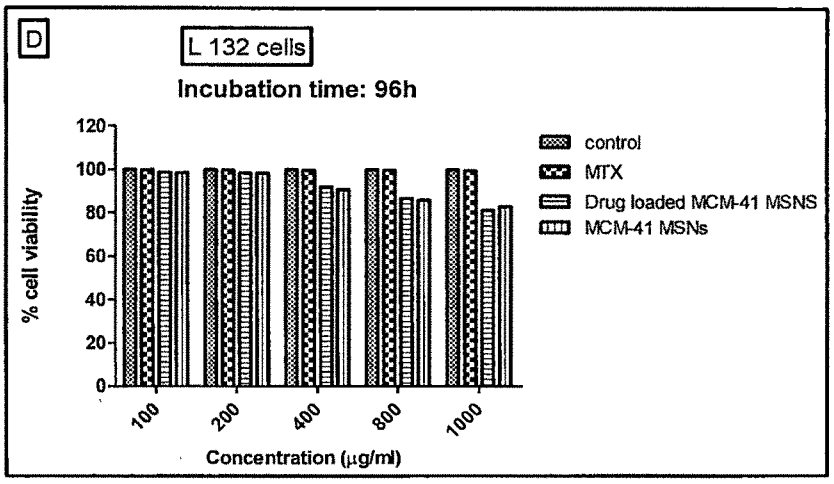
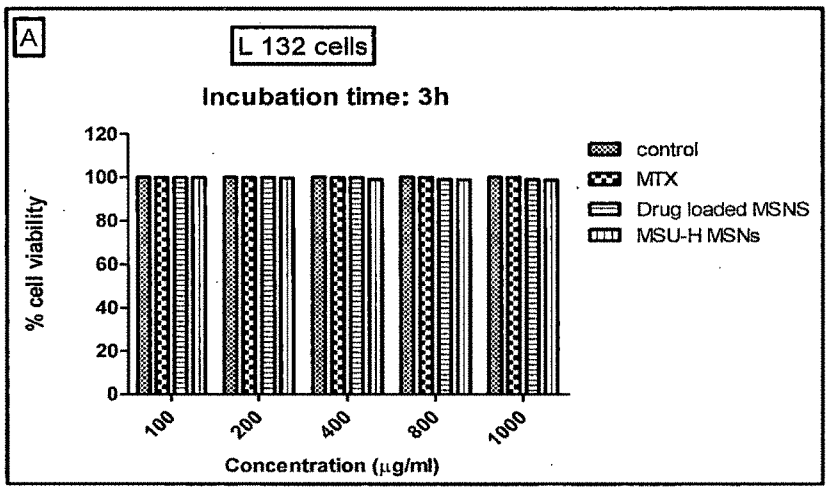


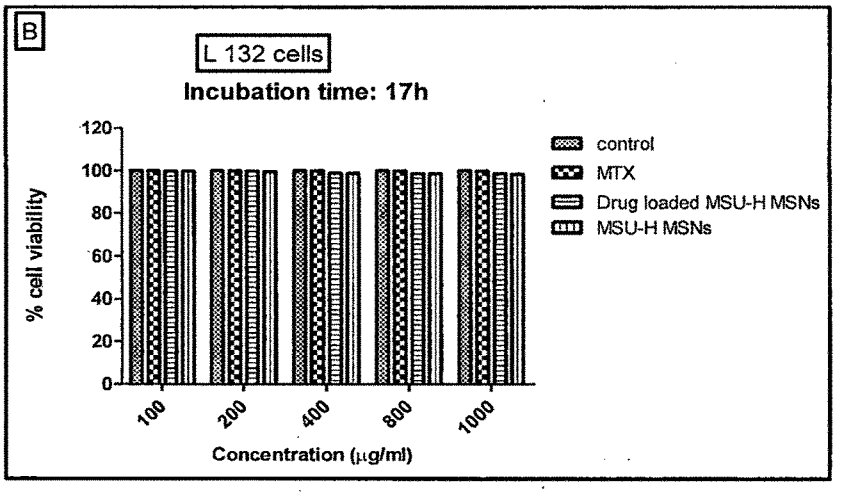
Figure 10.4: Effect of MTX + MCM-41 MSNs on cell viability of L-132 cells at different concentrations and incubation time (A-D)

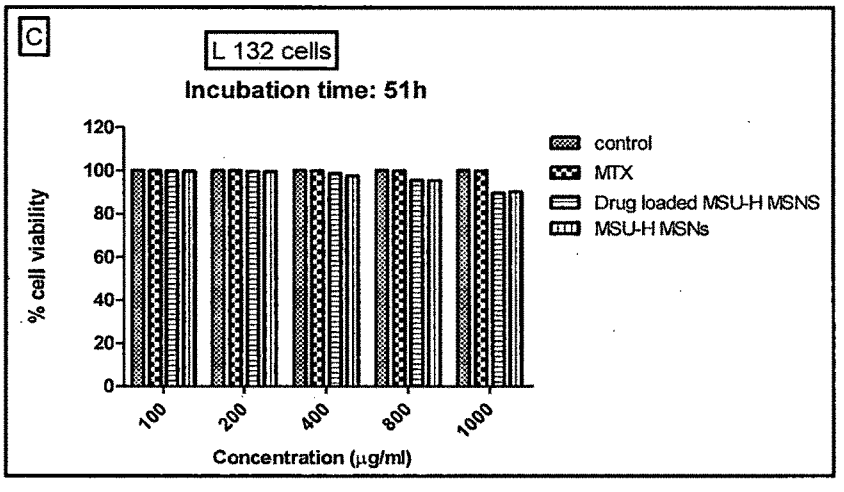
The cytotoxicity study on L-132 cells was performed for the MTX loaded MSU-H MSNs. The result of the study showed that MTX, MTX loaded MSU-H MSNs and MSU-H MSNs are devoid of significant toxic behavior to L-132 cells. The cell viability was checked at different incubation times (Fig. 10.5). The results obtained at each incubation time revealed that the viable cells are more than 85%. Percentage cell viability after the incubation period of 96h is shown in Table 10.4. It was observed that at concentrations up to 400 µg/ml, more than 97% of the cells are viable but in presence of high concentration (1000 µg/ml) of MTX loaded MSU-H and its placebo, the cell viability was more than 85%.

Table 10.4: Percentage viability of L-132 cells against MTX + MSU-H MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	MTX	MTX loaded MSU-H MSNs	MSU-H MSNs
100	99.91	99.53	99.57
200	99.88	99.40	99.24
400	99.79	98.41	97.87
800	99.75	90.67	89.54
1000	99.70	85.47	84.49


A
L 132 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MSNS
MSU-H MSNs


B
L 132 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MSU-H MSNs
MSU-H MSNs


C
L 132 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
MTX
Drug loaded MSU-H MSNS
MSU-H MSNs

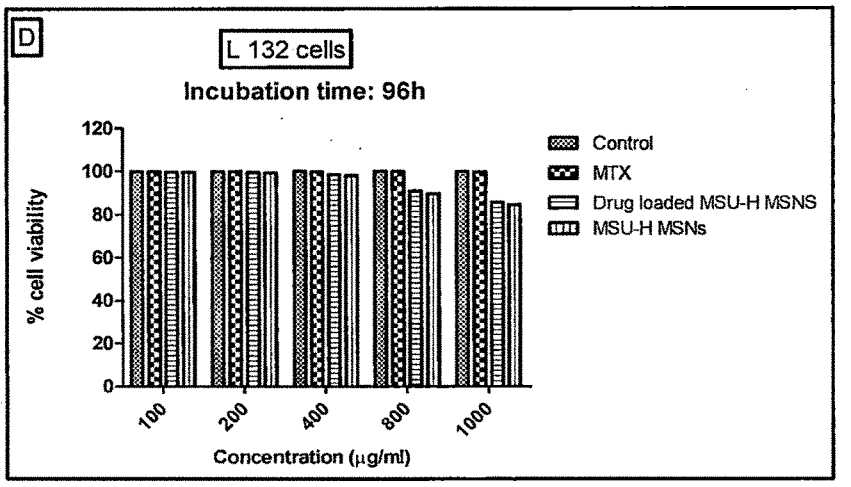


Figure 10.5: Effect of MTX + MSU-H MSNs on cell viability of L-132 cells at different concentrations and incubation time (A-D)

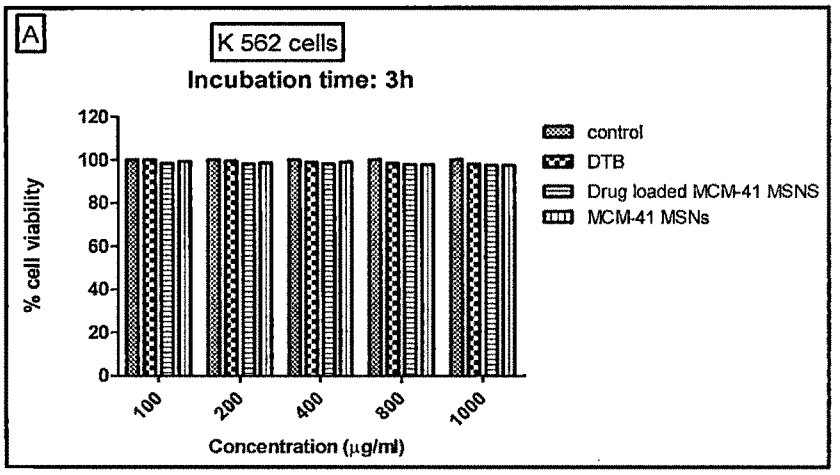
10.5.3 DTB loaded MSNs (MCM-41 and MSU-H) and K-562 cell line

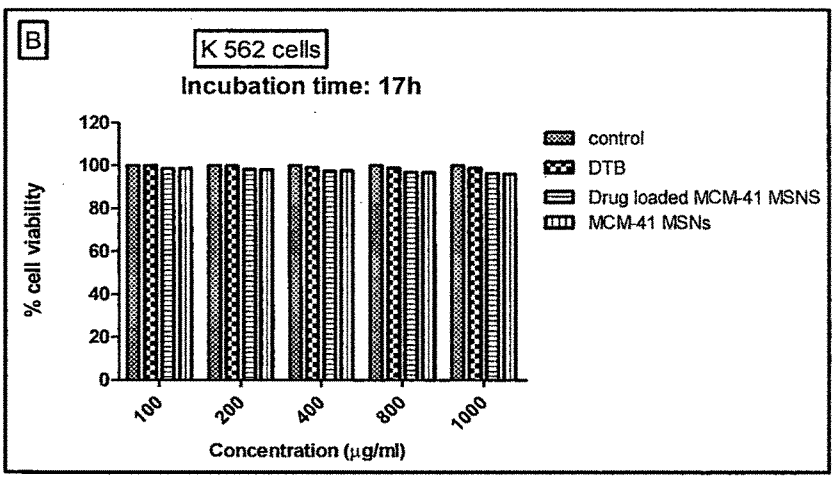
The cell cytotoxicity study also conducted for the second drug i.e. dasatinib. The study was performed with K-562 and L-132 cell lines.

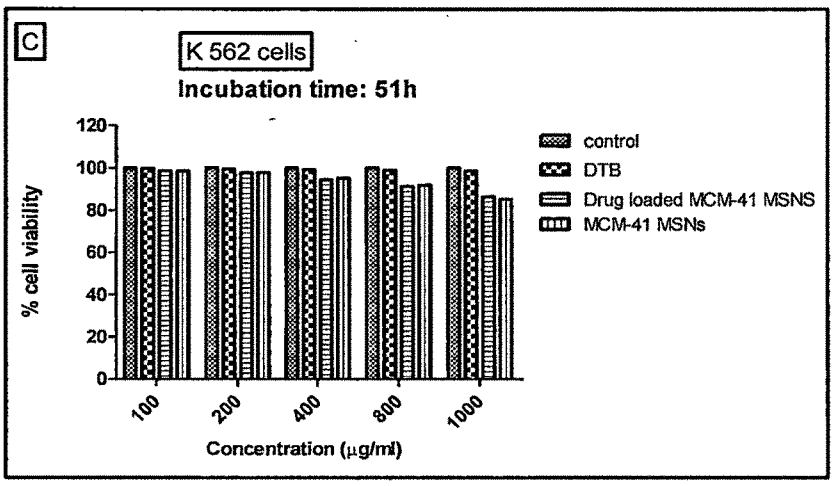
The cellular viability of K-562 cells was investigated over various concentrations of DTB and their MCM-41 formulation and/or placebo (MCM-41 MSNs). Fig. 10.6 shows the cell viability at different incubation times (3, 17, 51 and 96h). Table 10.5 represents the data of cell viability after the incubation time of 96h of DTB loaded MCM-41 and its placebo. It was found that at concentrations up to 400 µg/ml more than 88% of the cells are viable whereas at concentration of 1000 µg/ml of DTB loaded MCM-41 and its placebo showed decrease in cell viability to 77% and 76% respectively.

Table 10.5: Percentage viability of K-562 cells against DTB + MCM-41 MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	DTB	DTB loaded MCM-41 MSNs	MCM-41 MSNs
100	99.72	98.20	98.54
200	99.65	97.49	97.91
400	99.54	88.57	89.40
800	98.95	81.67	80.48
1000	98.77	77.74	76.79


A
K 562 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MCM-41 MSNS
MCM-41 MSNs


B
K 562 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MCM-41 MSNS
MCM-41 MSNs


C
K 562 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MCM-41 MSNS
MCM-41 MSNs

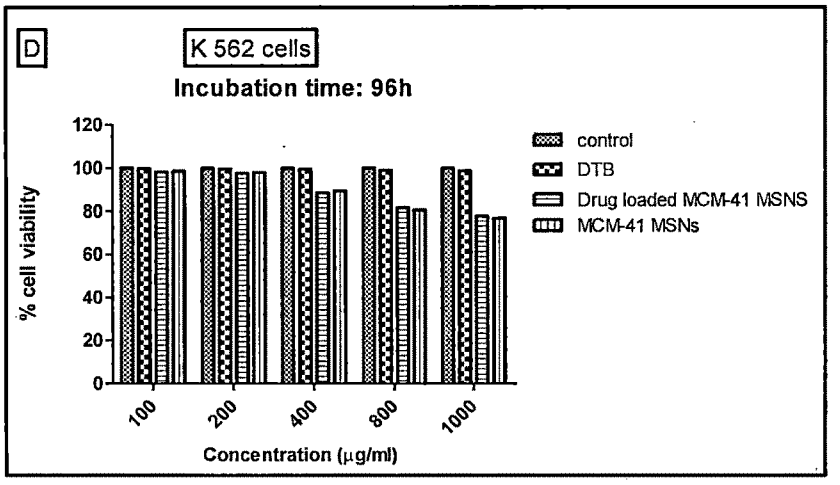
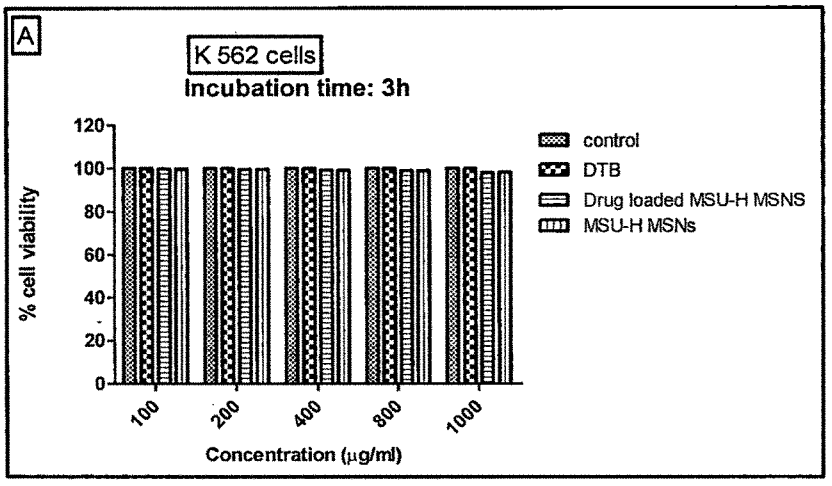


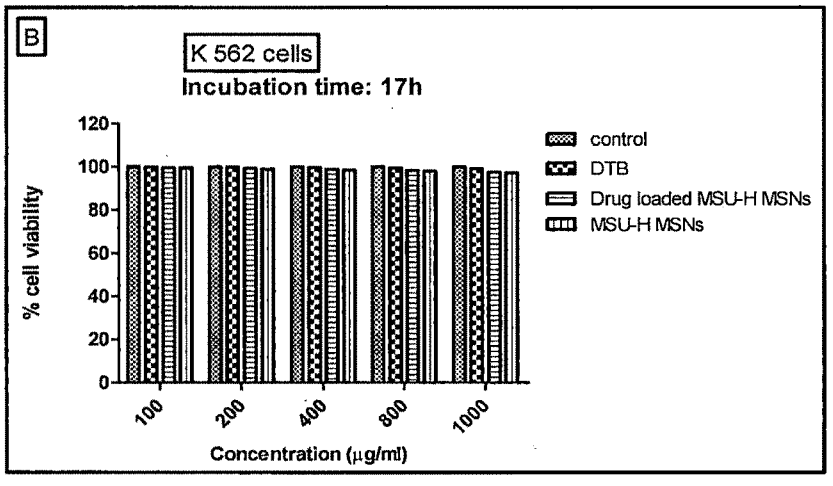
Figure 10.6: Effect of DTB + MCM-41 MSNs on cell viability of K-562 cells at different concentrations and incubation time (A-D)

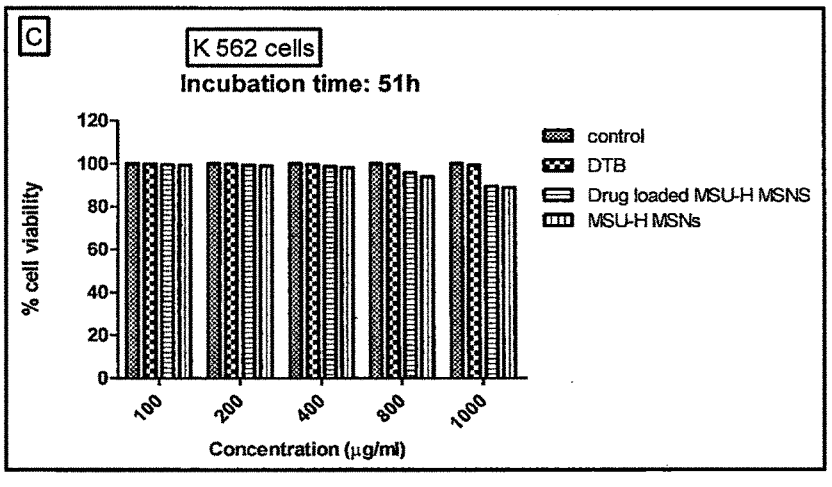
The results of cell cytotoxicity study on K-562 cells of DTB loaded MSU-H MSNs showed that DTB, DTB loaded MSU-H MSNs and MSU-H MSNs are significantly non toxic to K-562 cells. The cell viability was checked at different incubation of time (Fig. 10.7). The results obtained at these incubation times revealed that the viable cells are more than 85%. Percentage cell viability after the incubation period of 96h is shown in Table 10.6. It was observed that at concentrations up to 400 µg/ml, more than 96% of the cells were viable whereas at the concentration of 1000 µg of DTB loaded MSU-H and its placebo show the cell viability was more than 86%.

Table 10.6: Percentage viability of K-562 cells against DTB + MSU-H MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	DTB	DTB loaded MSU-H MSNs	MSU-H MSNs
100	99.65	99.35	99.24
200	99.54	99.10	98.17
400	99.41	98.47	97.86
800	99.37	92.62	91.27
1000	99.00	86.81	88.20


A
K 562 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MSU-H MSNS
MSU-H MSNs


B
K 562 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MSU-H MSNs
MSU-H MSNs


C
K 562 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MSU-H MSNS
MSU-H MSNs

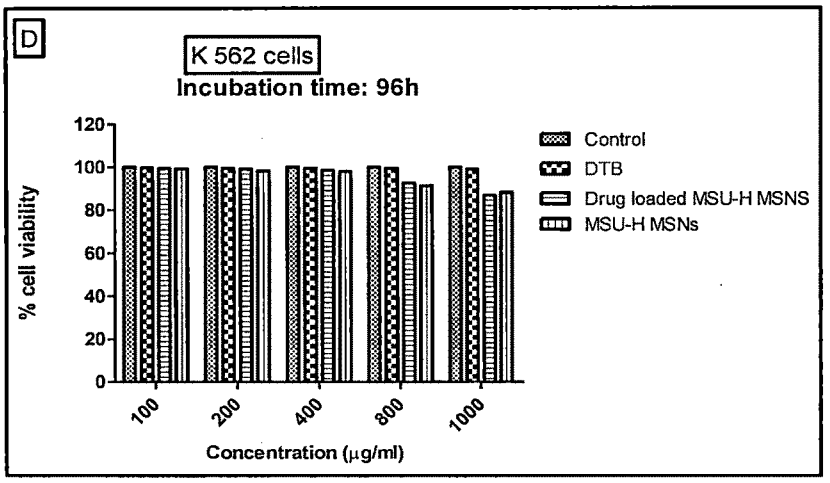


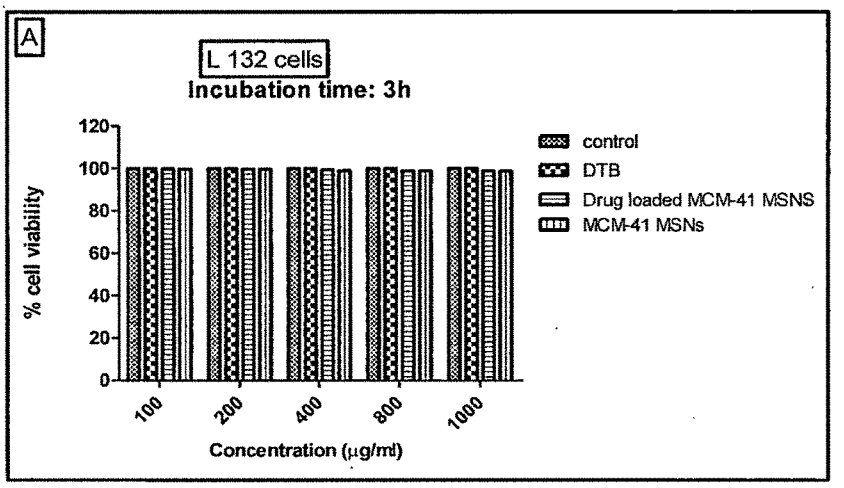
Figure 10.7: Effect of DTB + MSU-H MSNs on cell viability of K-562 cells at different concentrations and incubation time (A-D)

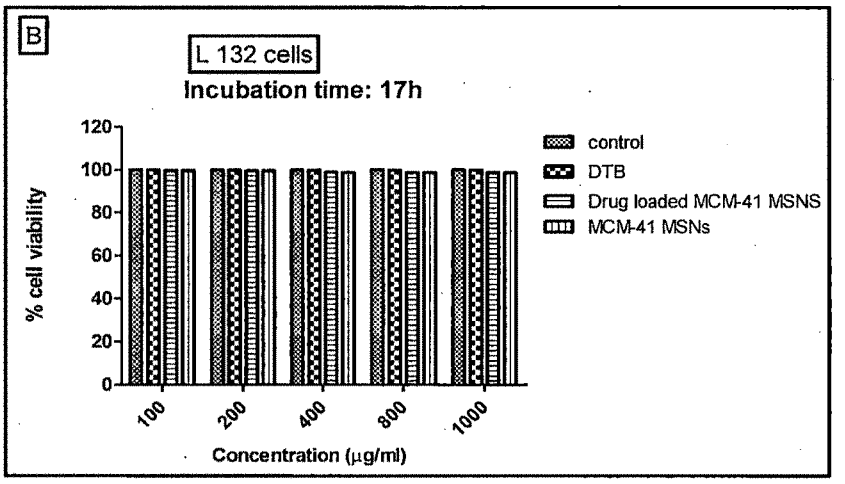
10.5.4 DTB loaded MSNs (MCM-41 and MSU-H) and L-132 cell line

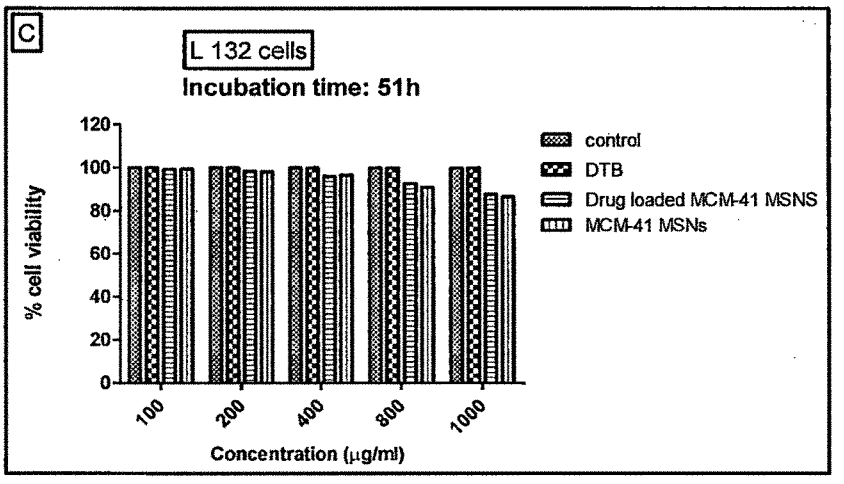
The cell viability of L-132 cells were investigated over various concentrations and incubation time for DTB, DTB loaded MCM-41 MSNs and MCM-41 MSNs. Graphically the cell viability of L-132 cells at different concentration and incubation times are shown in Fig. 10.8. The cytotoxicity assay result revealed that more than 80% cells are viable after 96h of incubation. Table 10.7 represent the data of cell viability after the incubation time of 96h of DTB loaded MCM-41 and its placebo, clearly revealed that MCM-41 MSNs alone and drug loaded MCM-41 MSNs were devoid of any significant cytotoxic effect. It was observed that concentrations up to 400 µg/ml about 90% of the cells are viable, whereas at the concentration of 1000 µg/ml of DTB loaded MCM-41 and its placebo, the cell viability was found to be more than 80%.

Table 10.7: Percentage viability of L-132cells against DTB + MCM-41 MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	DTB	DTB loaded MCM-41 MSNs	MCM-41 MSNs
100	99.76	98.81	98.40
200	99.65	98.56	98.37
400	99.58	89.90	90.64
800	99.54	83.71	82.73
1000	99.49	79.54	80.46


A
L 132 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MCM-41 MSNS
MCM-41 MSNs


B
L 132 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MCM-41 MSNS
MCM-41 MSNs


C
L 132 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MCM-41 MSNS
MCM-41 MSNs

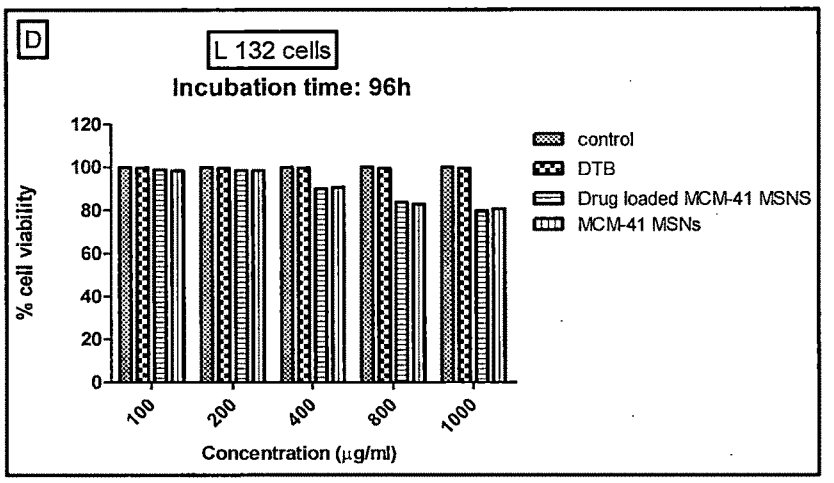
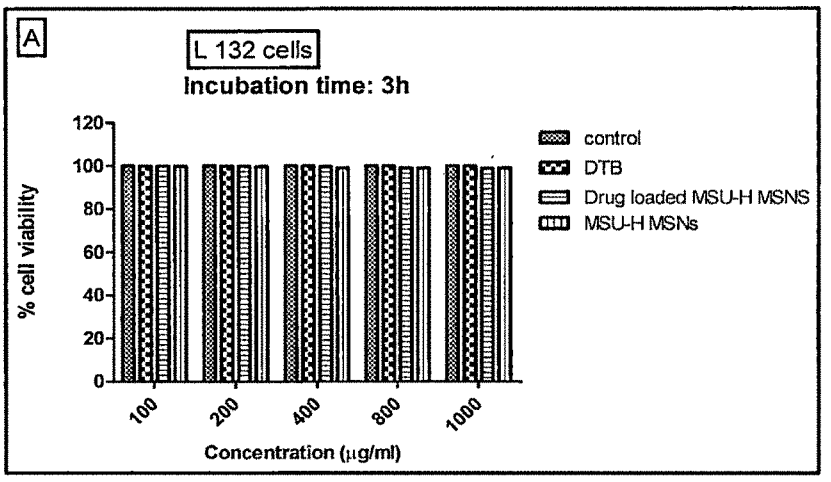


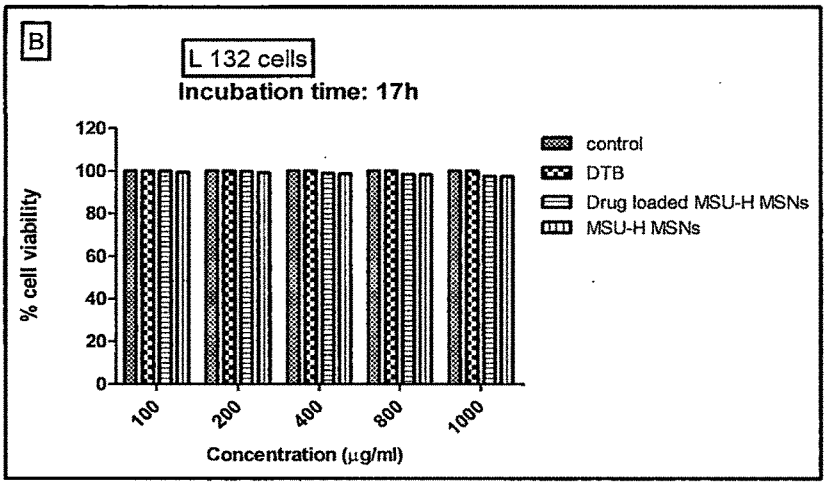
Figure 10.8: Effect of DTB + MCM-41 MSNs on cell viability of L-132 cells at different concentrations and incubation time (A-D)

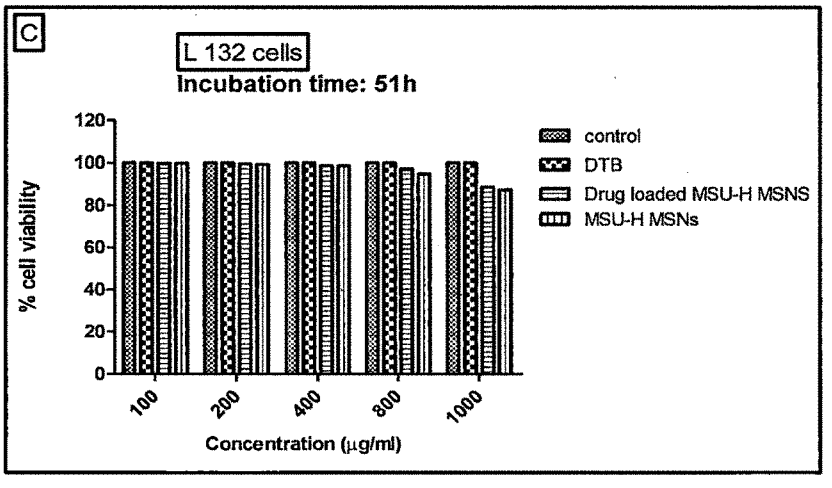
The cytotoxicity study on L-132 cells was performed and result showed that DTB, DTB loaded MSU-H MSNs and MSU-H MSNs are devoid of significant toxic behavior to L-132 cells. The cell viability was checked at different incubation of time (Fig. 10.9). The results obtained at each incubation time revealed that the viable cells are more than 85%. Percentage cell viability after the incubation period of 96h was shown in Table 10.8. It was observed that concentrations up to 400 µg/ml more than 97% of the cells are viable. Nevertheless, in presence of high concentration (1000 µg/ml) of DTB loaded MSU-H and its placebo, show the cell viability more than 85%.

Table 10.8: Percentage viability of L-132cells against DTB + MSU-H MSNs incubated up to 96h

Concentration (µg/ml)	% Cell viability		
	DTB	DTB loaded MSU-H MSNs	MSU-H MSNs
100	99.82	99.59	99.68
200	99.75	99.50	99.58
400	99.69	98.51	97.97
800	99.65	91.96	90.15
1000	99.58	88.58	85.36


A
L 132 cells
Incubation time: 3h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MSU-H MSNS
MSU-H MSNs


B
L 132 cells
Incubation time: 17h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MSU-H MSNs
MSU-H MSNs


C
L 132 cells
Incubation time: 51h
% cell viability
120
100
80
60
40
20
0
100
200
400
800
1000
Concentration (µg/ml)
control
DTB
Drug loaded MSU-H MSNS
MSU-H MSNs

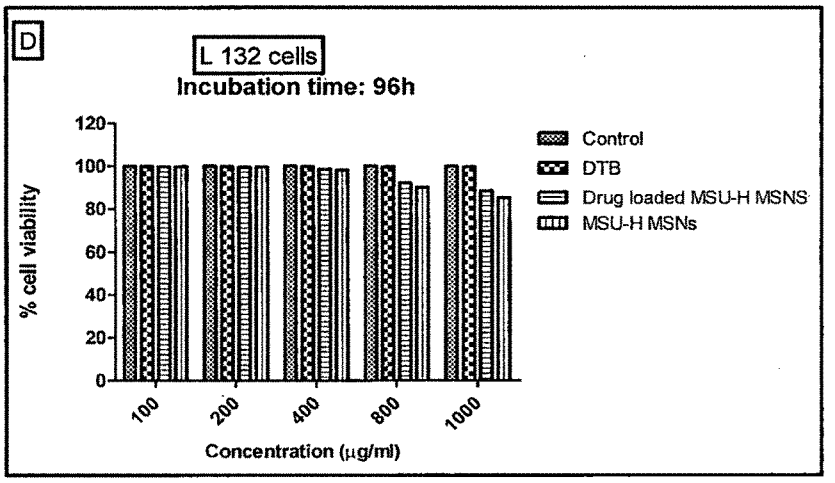


Figure 10.9: Effect of DTB + MSU-H MSNs on cell viability of L-132 cells at different concentrations and incubation time (A-D)

It is well documented that the cytotoxic behavior of MSNs depends on many factors, mainly include shape, size, concentration, surface morphology and chemistry of MSNs and type of cell culture as well as on the duration of exposure. Reduction in the cell viability was observed with all MSNs when certain threshold concentrations were reached.

The cell viability of both the MSNs was compared in K-562 and L-132 cells after incubation of 3h and 96h (Fig. 10.10 to 10.13). After the 96h of incubation of MTX loaded MCM-41 MSNs with K-562 cells, it was found that more than 81% of cells were viable whereas more than 86% of cells are viable with MTX loaded MSU-H MSNs (Fig. 10.10). The L-132 cells show the cell viability of 81% and 85% for the MTX loaded MCM-41 MSNs and MSU-H MSNs respectively (Fig. 10.11).

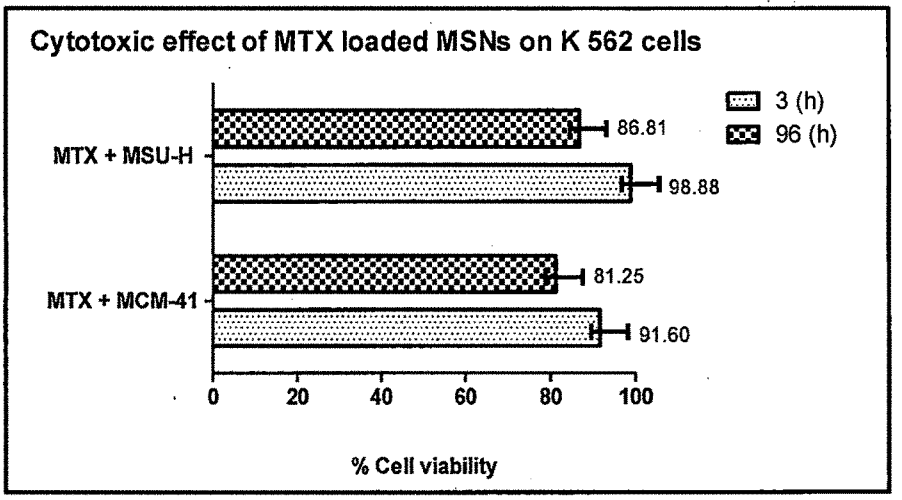


Figure 10.10: Effect of MTX loaded MSNs on cell viability of K-562 cells at 1000 µg/ml and 3 & 96 h of incubation time

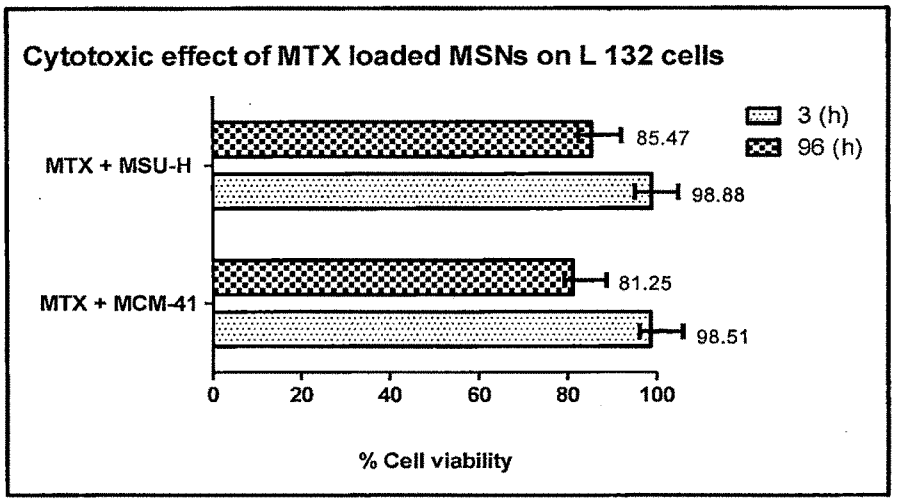


Figure 10.11: Effect of MTX loaded MSNs on cell viability of L-132 cells at 1000 µg/ml and 3 & 96 h of incubation time

Similarly after the 96h of incubation of DTB loaded MCM-41 MSNs with K-562 cells, more than 77% of cells were viable whereas more than 86% of cells are viable with MSU-H MSNs (Fig. 10.12). The L-132 cells show the cell viability of 79% and 88% for the MCM-41 MSNs and MSU-H MSNs respectively (Fig. 10.13). It can be concluded from cytotoxicity assay results that the MCM-41 and MSU-H MSNs are significantly non toxic to the selected cell lines. The numerical data of the assay results indicates that the most pronounced decrease in the cell viability of both the cell culture was when MSNs were present at higher concentrations and for longer duration of incubation time.

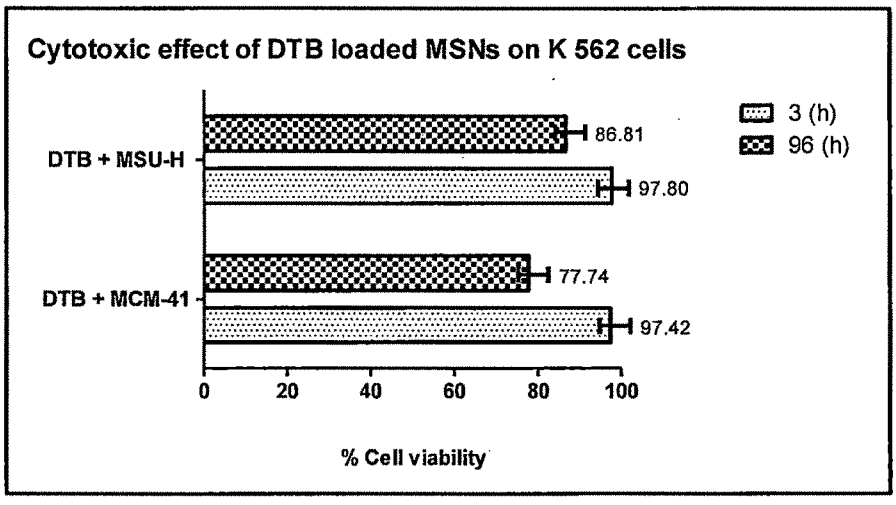


Figure 10.12: Effect of DTB loaded MSNs on cell viability of K-562 cells at 1000 µg/ml and 3 & 96 h of incubation time

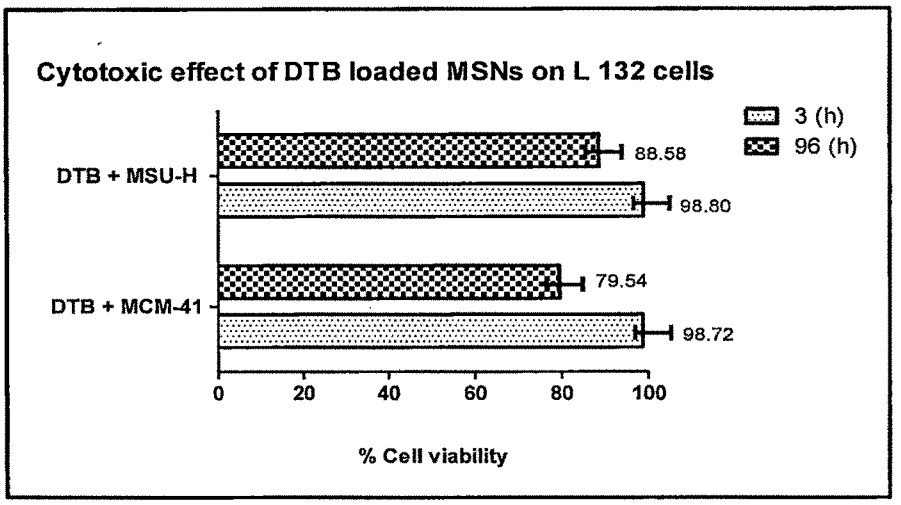


Figure 10.13: Effect of DTB loaded MSNs on cell viability of L-132 cells at 1000 µg/ml and 3 & 96 h of incubation time

Amongst the different factors; the chemistry of MSNs largely contributes to the viability of cells of the selected cell lines. It was found that cell viability number is more in case of MSU-H MSNs as compare to MCM-41 MSNs. The reason of decrease in cell viability might be the cationic surfactant; cetyl trimethyl ammonium bromide, used for synthesis. The traces of cationic surfactant in MCM-41 MSNs might be responsible for the toxicity of MCM-41 MSNs[39-44].

The surface area and particle size of the MSNs are the other factors which affects the cell viability. The surface area of MCM-41 and MSU-H was found to be 745 m^2/g and 644 m^2/g respectively. The average particle size of MCM-41 and MSU-H was found to be 100-150 nm[45-51] and 200-300 nm[52-57] respectively. It was found that the small size and large surface area MSNs facilitates more contact with cells than the larger size and lower surface area MSNs[58-66] which may explain the decrease in cell viability when MCM-41 MSNs was used.

10.6 Conclusion

The *in vitro* cytotoxicity of drugs loaded MCM-41 and MSU-H MSNs on K-562 and L-132 cells as a function of concentration and incubation time was demonstrated. The main difference between the MCM-41 and MSU-H MSNs studied was their surface morphology and chemistry properties. The results provide clear evidence that both the MSNs were having different surface morphology and chemical properties induce different *in vitro* cytotoxic responses. The decrease in cell viability of selected cells was more prominent when small size and large surface area MSNs exposed to higher concentration for longer duration of incubation. Under the experimental conditions tested it was found that, the both the MSNs were non toxic when used at concentration of 1000 µg/ml and 96h of incubation time.

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