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IN VITRO EVALUATION OF METHOTREXATE LOADED WITH SOLID LIPID NANOPARTICLES FOR BREAST CANCER

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ABSTRACT

Breast cancer is cancer that develops from breast tissue lump include in breast cancer, change in breast shap and skin is dimpling, it is the most common type breast cancer is ductal carcinoma. The present study is designed to methotrexate loaded with solid lipid nanoparticles (slns) to target Breast cancer. The developed nano-carriers where characterized with respect to particle size, PDI, zeta potential, drug loading and entrapment, in vitro releas etc. The characterized formulation were used to comparatively assess cellular uptake, cell-viability, apoptosis, lysomal membrane permeability, bioavailability, biodidtsribution, changes in tumer volume in anumal survival. The results ex-vivo showed greater cellular uptake and better cytotoxicity at lower IC50 of methotrexate in breast cancer cells. Further, we observed increased programmed cell death (apoptosis) with altered lysosomal membrane permeability and better rate of degradation of lysosomal membrane in-vitro. On the other hand, in-vivo evaluation

showed maximum bioavailability and tumor targeting efficiency with minimum secondary drug distribution in various organs with formulated and anchored nano-carrier when compared with free drug. Moreover, sizeable reduction in tumor burden was estimated with fucose decorated SLNs as compared to that seen with free MTX and SLNs-MTX. Fucose decorated SLNs showed promising results to develop therapeutic interventions for breast cancer, and paved a way to explore this promising and novel nano-carrier which enables to address breast cancer.

KEYWORDS: Brest cancer, Methotrexate, Solid lipid nano particle. Targeted delivery.

1. INTRODUCTION

BREAST CANCER

Breast cancer is one of the world's most devastating diseases commonly found in women of western countries. It begins in breast tissue, usually the ducts and lobules. Based upon the evidence of metastasis, it is called prostate cancer which originates from breast tissue. Breast cancer shows no symptoms in the early stages; therefore screening tests often fails to detect the disease at this time. Following changes may occur with breast cancer growth such as A lump in or near the breast, a change in the size or shape of the breast, a changes in the skin of the breast, areola or nipple. The causes of breast cancer are still unknown, but there is a combination of risk factor including life style factor, environmental factor, genetic factor, and hormonal factor that may be responsible for the breast cancer. Breast cancer is a heterogeneous disease which depends on the expression level of progesterone receptor, estrogen receptor, and HER-2/neu receptor (human epidermal growth factor receptor-2). The molecular biology of the breast cancer is complex as multiple factors contribute in the development of breast cancer such as genetic mutation in BRCA1, BRCA2 and p53 and cross- talk between different signaling pathways. Cell-signaling pathways allow normal programs of proliferation, transcription, growth, migration, differentiation and death in the normal cell. But in the case of breast cancer cell, these normal programs are altered by altering cell signaling pathway. Various signaling pathways that play an important role in development and progression of breast cancer are initiated by the interaction between growth factors and their receptor, such as human epidermal growth factor receptor (HER-2 and VEGF) and their ligands, as well as insulin-like growth factor (IGF) and IGF-IR. There are many types of stages in breast cancer are as follows.[1]

(1) Stage 0: it consists of three types of breast carcinoma.

(2) Stage I: It is divided into two stages Ia and Ib.

(3) Stage II: It is divided into Stage IIa and Stage IIb.

(4) Stage III: it is divided into IIIa and IIIb.

(5) Stage IV: cancer has spread to other parts of the body, mostly to lungs, bone or liver.

1.2 Pathophysiology of breast cancer

A damaged DNA is the main cause of uncontrolled cell growth and beginning of carcinoma. For most of the damaged DNA, cell itself do repair mechanism but in the case of carcinoma condition the situation alter, the DNA unable to restore the damage which is circularized to newer abnormal cell born from the defective cell. The improper functioning of cell growth and cell division pathway with normal cells which is the intermediate pathway of stromal cell and epithelial cells which leads to the breast cancer condition. A genetic mutation which is one of the reasons of the DNA damage leads the cells over exposure estrogen that causes breast carcinoma condition. Not only the mutation but individuals inherits damages genes such as BRCA1,BRCA2, P53, mTOR and HER2 among others are the reasons behind the breast cancer to some individuals. Family history also plays one of the key factors for developing the risk of the breast cancer.[2]

MATERIALS AND METHODS

5.1 Materials

Table no 1: Chemical/Reagents Required: Chemicals was used in the proposed work, as shown.

S.No.	Name of Chemicals	Company
1	Ethanol	LOBA chem
2	Chloroform	LOBA chem
3	Ether	LOBA chem
4	HCL	LOBA chem
5	Potassium dihydrogen phosphate	LOBA chem
6	NaOH	LOBA chem
7	n- Octanol	Sigma Aldrich
8	Glyceryl monosterate	LOBA chem
9	Soya lecithin	Sigma Aldrich
10	Methanol	LOBA chem
11	Dichloromethane	Sigma Aldrich
12	Tween 80	LOBA chem
13	Dialysis tube	Himedia, India
14	Acetonitrile HPLC grade	Sigma Aldrich
15	Water HPLC grade	LOBA chem
16	Heparin solution	Sigma Aldrich

Instruments

Instruments/ Apparatus required: Instruments was used in the proposed work shown.

Table no 2: (List of Instruments & there company name).

S.No.	Instruments name	Company name
1	Magnetic stirrer	Remi, India
2	Digital Weight	Balance Keroy, India
3	Centrifuge	Remi, India
4	Zetasizer	Malvern Instruments Ltd.
5	UV- Spectroscopy	Shimadzu 1800
6	Infrared spectrophotometer	Shimadzu IR Affinity 1
7	Probe sonicator	PCI
8	Gradient HPLC system	Youngling instrument
9	Lyophilizer	Multitech scientific instrument lab

5.2 METHOD

5.2.1 Melting Point

The melting point was determined by melting point apparatus (Open Capillary Method). The chemical was taken in capillary tubes which was closed by one side and was placed in the melting point determining apparatus. The temperature was gradually increased automatically. The temperature (by using a thermometer) at which sample was melted was noted.[20]

5.2.2 Solubility Studies

The solubility of methotraxate was tested in different solvents such as ethanol, chloroform, ether, PBS etc. A semi quantitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity o solute or vice versa. After each addition, the system is vigorously shaken and examined visually for any un-dissolved solute particles. The solubility was observed only by the visual inspection.[20]

5.2.3 Determination of λmax for Methotraxate

The standard stock solution of methotraxate was prepared by dissolving 10mg of drug in 10ml of phosphate buffer (pH 7.4) further volume was made up to 100ml with buffer. From this solution 10ml was pipetted out and transferred into a 100ml volumetric flask, volume was made up to 100ml with respective buffer and from this solution pipetted out 10ml in a test tube and subjected for UV scanning in the range 200-400nm. The λmax was found to be 303nm in 7.4 pH PBS and 307nm in 0.1N HCL solution.[21]

5.2.4 Calibration curve

Accurately weighed 100mg of MTX was dissolved in phosphate buffer (pH 7.4) in a 100ml volumetric flask, diluted with buffer up to the volume. 20ml of this solution was pipetted out into a second 100ml volumetric flask; buffer was added to make up the volume. A set of standard dilution of 5, 10, 15, 20 and 25µg/ml of drug were prepared by transferring aliquots of 0.2, 0.4, 0.6, 0.8, and 1ml of stock solution in 10ml volumetric flask and volume makeup to the mark with medium. The absorbances of resulting solution were measured at the wavelength of maximum absorbance using phosphate buffer (pH 7.4) as a blank.[21]

5.2.5 Partition Coefficient

Equal volumes (10ml) of buffer (pH 7.4) and n- octanol were added to a 50ml stoppered separating funnel containing 10mg of a drug. The mixture was kept for 24hrs at room temperature with intermittent shaking. The two phases were separated and the aqueous was analyzed spectrometrically for drug content. The partition coefficient was calculated by[21]

Ko/w =Coil/Cwater.

5.3 Formulation of Solid Lipid Nanoparticles

5.3.1 Preparation of Solid Lipid nanoparticles

5.3.1.1 Solvent emulsification evaporation method

The Solid Lipid Nanoparticles were prepared and characterized by reported method of Dinda. A *et.al*. with minor modification. MTX loaded SLN were prepared by using glyceryl monosterate (GMS) as a lipid, soya –lecithin as co-surfactant and tween 80 as a surfactant. Solvent evaporation method was selected to prepare SLNs due to convenience of lab scale equipments and suitability of the method. In this method, accurately weighed amount of 60mg lipid, 25mg drug and 20 mg co-surfactant were dissolve in 1ml of organic solvent which in ratio of 2:8 (methanol: dichloromethane). In 50 ml beaker 10 ml of 1% w/v tween 80 solutions is taken. Then organic phase were added to this 10ml of 1% tween 80 solutions and homogenized at 12000 rpm for 3 min which result in coarse emulsion.[21, 22]

These coarse emulsions were subjected to ultra –sonication for 10 min by using probe sonicator. During sonication, due to solvent emulsification and evaporation SLNs are precipitated and settle down. MTX loaded SLNs were formed and collected, stored at cold temperature and characterized sample.[21]

5.4 Characterization of Solid Lipid Nanoparticles

5.4.1 Particles Size & Morphology Characterization of MTX loaded SLNs

Characterizations of SLNs are primarily evaluated by the particle size distribution and morphology. With the aid of electron microscopy it's now possible to ascertain the morphology as well as the size of SLNs. Application of SLNs in drug release and drug targeting can be conveniently determined by various tools. It has already been reported that particles size of SLNs has profound effect on the drug release. Smaller the size of SLNs larger surface area, which results in to fast drug release. Loaded drug when exposed to the particle surface area causes significant drug release. In contrast, inside the SLNs drugs slow diffusion of larger particles occurs. Consequently smaller particles tend to aggregate during storage and transportation of SLNs dispersion. Therefore, there is a mutual compromise between maximum stability and small size of SLNs. In addition degradation of the polymer can also be affected by the particle size. Particles size and polydispersity index of samples were analysed by a particles size analyzer (Malvern instrument), instrument using DLS technique. The particles morphology of the SLNs were studies by SEM. Scanning electron microscopy (SEM) is used for the morphological analysis of MTX loaded SLNs. Approximately 2-5µl of the SLN was placed on the glass plate and dried at room temperature. After drying, the sample was coated with gold metal to a thickness of 10nm under vacuum and then examine.[22]

5.4.2 Encapsulation efficiency and Drug loading

The encapsulation efficiency (EE) and loading capacity (LD) of SLNs were determined by centrifugation method. The SLNs dispersions were ultra-centrifuge at 15000 rpm for 20min at 4ºC in a refrigerator centrifuge to collect supernatant liquid. The collected liquid was filtered to measure the free drug concentration after suitable dilution with phosphate buffer pH 7.4. MTX concentration were measured in the supernatant by UV spectroscopy at 307nm.The concentration of free drug MTX in the supernatant was obtained by comparing the absorption of the supernatant to the standard curve related to absorption and MTX concentration. The encapsulation efficiency (EE) and loading capacity (LD) were calculated by the following equation.[22]

$$\textbf{EE (\%) = amount of MTX for preparing formulation – amount of MTX in supernatant amount of MTX for preparing formulation} \times 100$$

DL (%) = amount of MTX for preparing formulation – amount of MTX in supernatant
Polymer weight + amount of MTX for preparing formulation×100

5.5 *In vitro* Study

5.5.1 Drug release

It's very essential to determine extent of the drug release and in order to obtain such information most release methods require that the drug and its delivery vehicle be separated. Drug loading capacity of the nanoparticles is defined as the amount of drug bound per mass of polymer or in another term it is the moles of drug per mg polymer or mg drug per mg polymer or it could also be given as percentage relative to the polymer. Various techniques such as UV spectroscopy or high performance liquid chromatography (HPLC) after ultracentrifugation, ultra filtration, gel filtration or centrifugal ultra filtration are used to determine this parameter.[23] Methods that are employed for drug release analysis are also similar to drug loading assay which is more often assessed for a period of time to evaluate the drug release mechanism. In vitro drug release studied were performed by dialysis beg diffusion method. An accurately weigh amount of MTX loaded SLNs dispersion was transferred to dialysis beg and sealed. The SLNs loaded dialysis beg was incubated in 50ml PBS of pH 7.4 which is used as sink solution and stirred at a constant speed of 50 rpm at 37ºC ± 0.5ºC. Aliquots of 2ml of released media were taken at specific interval of 0.5, 1, 2, 3, 4, 8, 12 and 24 hrs and equivalent volume of fresh PBS was replaced to keep the volume of the system identical. Then the drug content was determined by UV spectroscopy by measuring the absorbance at 307nm.[24]

5.6 *In vivo* Studies

5.6.1 Pharmacokinetic Study

The pharmacokinetic parameters were determined by using Swiss albino mice of either sex, age group of 5-6 weeks and weighing in between 22-25gm. The animal was fed with a standard pellet diet and water adlibitum. The animal was divided into two groups. Each group contain 3 animals. Group I were treated with MTX loaded SLNs (equivalent to 2.5mg/kg) whereas, Group II were treated with plain MTX solution (2.5 mg/kg). Both were diluted with pH 7.4 PBS and administered intraperitoneally. The blood samples were collected from retro orbital plexus using capillary tube at predetermined time intervals and put into heparin solution. The serum samples were separated from heparinised blood, by centrifuging at 600rpm in a cooling centrifuge and the serum levels of the drug was determined by HPLC.

The mobile phase was 8:92 (Acetonitrile: Buffer), the detector wavelength 307nm and the flow rate was 1.4ml/min.[25]

5.6.2 Biodistribution Study

For distribution study, Swiss albino mice (2-3 month old o either sex) weighing 22-30gm were taken. The animals were reared on laboratory chew pallets, fed adlibitum and had free access to food water at all time. The room was maintained at 25± 2ºC with natural daylight and no light after 12 hrs until morning. The animals are assign in two groups, each group consist of six mice.[26] Group I treated with Plain MTX drug and Group II were treated with MTX loaded SLNs. MTX and prepared SLNs were injected intravenously through the tail vein method with dose 30mg/kg. Mice from each group were sacrificed 24 hr after administration of samples, and various organ (Brain, lungs, liver, kidney, heart, blood were isolated and homogenized with 30ml PBS pH7.4. The homogenized organs were centrifuged at 6000 rpm for 15min, and the supernatant was collected and analyzed for drug content UV spectrophotometer at 307nm.[27,28]

RESULTS AND DISCUSSION

6.1 Identification of Chemicals

6.1.1 Preformulation Study

Methotraxate

6.1.1.1 Physical appearance: The physical appearance of methotraxate was analyzed by visually observation.

Table no 3: Physical appearance.

Parameter	Observation
Physical appearance	Odourless yellow to orange- brown crystalline powder

6.1.1.2 Melting point

The melting point was determined by melting point apparatus (open capillary method). The chemical was in capillary tube which was closed by one side and was placed in the melting point determining apparatus. The temperature was gradually increased automatically. The temperature (by using thermometer) at which sample was melted was noted.

Table no 4: Melting point of methotrexate.

Name of drug	Melting Point
Methotraxate	180ºC to 189ºC

6.1.1.3 Solubility study

Solubility may be defined as the spontaneous interaction of two or more substances to form a homogenous molecular dispersion. The solubility of MTX was tested in various solvents. A definite quantity (25mg) of chemical was dissolved in 10ml of each investigated solvents at room temperature.

Table no 5: Solubility of MTX in different solvents.

S.No.	Solvents	Solubility
1	Water	Insoluble
2	Ethanol	Insoluble
3	Chloroform	Insoluble
4	Ether	Insoluble
5	0.1N HCL	Soluble
6	PBS of 7.4 pH	Soluble

6.1.1.4 Determination of λmax

The absorption maximum (λmax) of Chemical was found to be 307nm in phosphate buffer pH 7.4 and 310nm in 0.1N HCL determined by UV visible spectrophotometer.

Table no 6: Wavelength of maximum absorption of MTX.

Solvent	Λmax
Phosphate buffer solution 7.4pH	307nm
0.1N HCL	310nm

6.1.1.5 Determination of standard calibration curve

Standard calibration curve in table, absorbance of MTX at different concentration 5, 10, 15, 20 and 25 µg/ml in different solvent i.e. Phosphate buffer pH 7.4 and 0.1N HCL respectively. Graph shown linear absorbance and correlation coefficient found to be 0.991 and 0.9989 respectively.

Table no 7: Calibration curve of MTX in PBS pH 7.4.

Concentration (µg/ml)	Absorbance (nm)	Statistical parameter
5	0.302	Y= mx + c Y= 0.031x + 0.128 r^2= 0.991
10	0.433	
15	0.558	
20	0.751	
25	0.929	

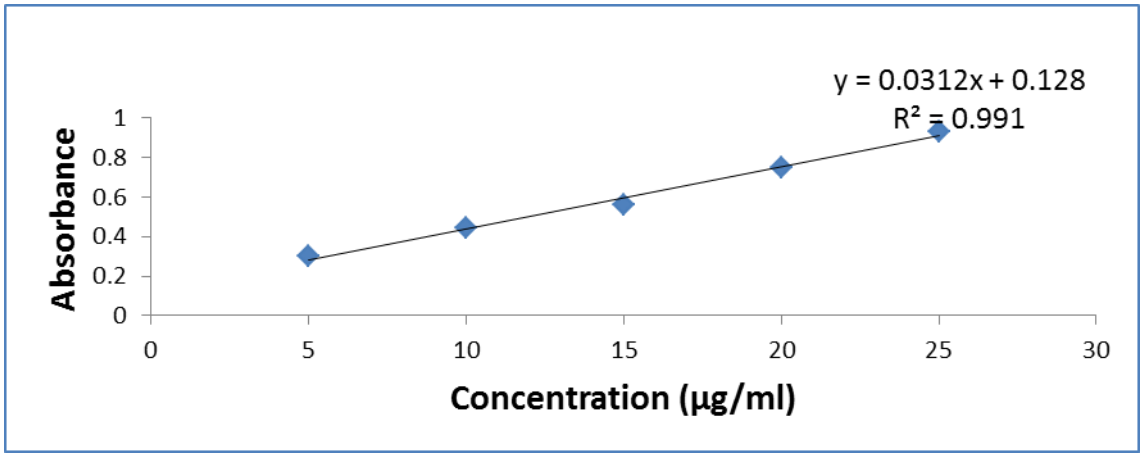

Figure no 9: Calibration curve of MTX in phosphate buffer pH 7.4.

Table no 8: Calibration curve of MTX in 0.1N HCL.

Concentration (µg/ml)	Absorbance (nm)	Statistical parameter
5	0.031	Y= mx +c Y= 0.0053x + 0.0039 r²= 0.9989
10	0.056	
15	0.081	
20	0.111	
25	0.135	

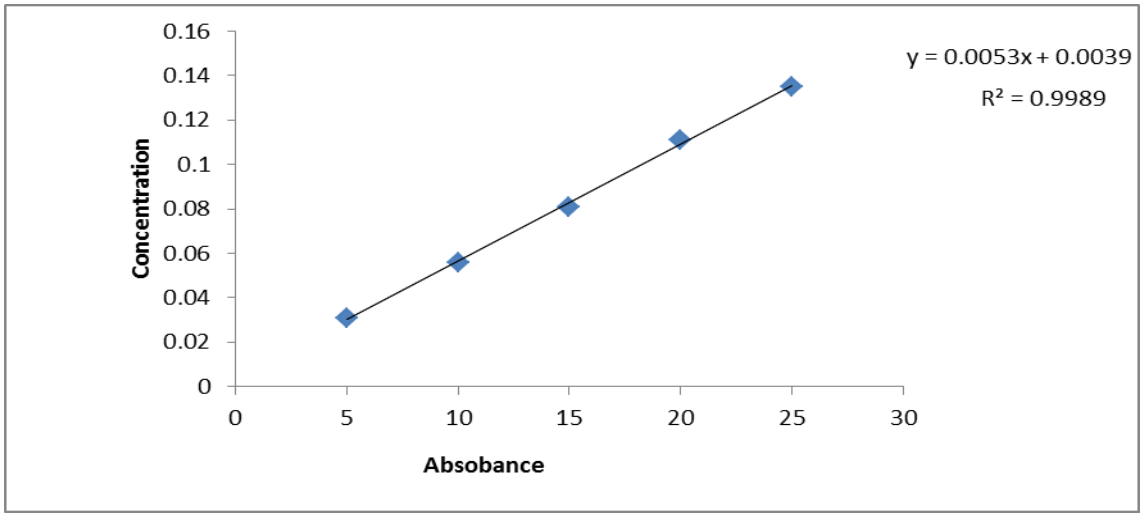

Figure no 10: Calibration curve of MTX in 0.1N HCL.

6.1.1.6 Partition coefficient

Equal volumes (10ml) of buffer (pH 7.4) and n- octanol were added to a 50ml stoppered separating funnel containing 10mg of a drug. The mixture was kept for 24hrs at room temperature with intermittent shaking. The two phases were separated and the aqueous was analyzed spectrometrically for drug content. The partition coefficient was calculated by.

Ko/w =Coil/Cwater

Table no 9: Partition coefficient of MTX.

Aqueous phase	Organic phase	Methotraxate		
		Aqueous	Oily	Po/w
Phosphate buffer of pH 7.4	n- Octanol	4.56	1.12	0.24

6.1.2 Characterization of SLNs

6.1.2.1 Particle size & morphological evaluation of MTX loaded SLNs

With optimizing synthesis process, MTX loaded SLNs observed the mean size of 120nm, very narrow size distribution (1 ± 0.01). SLNs were investigated by SEM and the resulted that the MTX loaded SLNs were spherical in shape, the mean diameter was approximately 40nm and average size was about 120nm. These particles have mean diameter 98.1 ± 7.5nm for drug delivery purpose which shows high potential for permiability due to more diffusion.

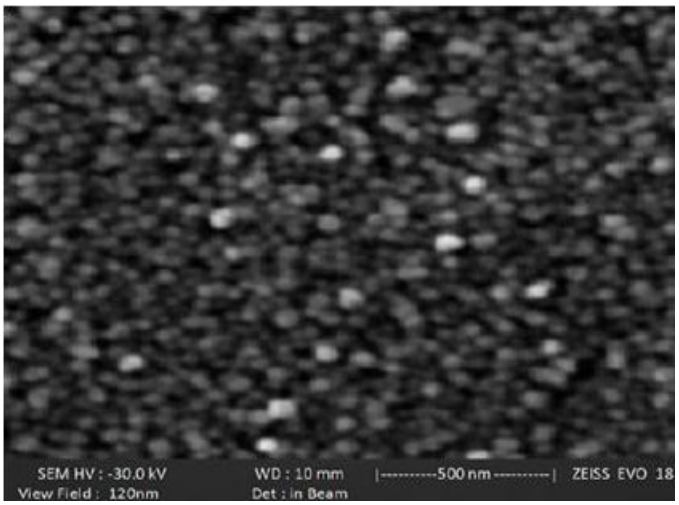

Figure 11: SEM of MTX loaded SLNs.

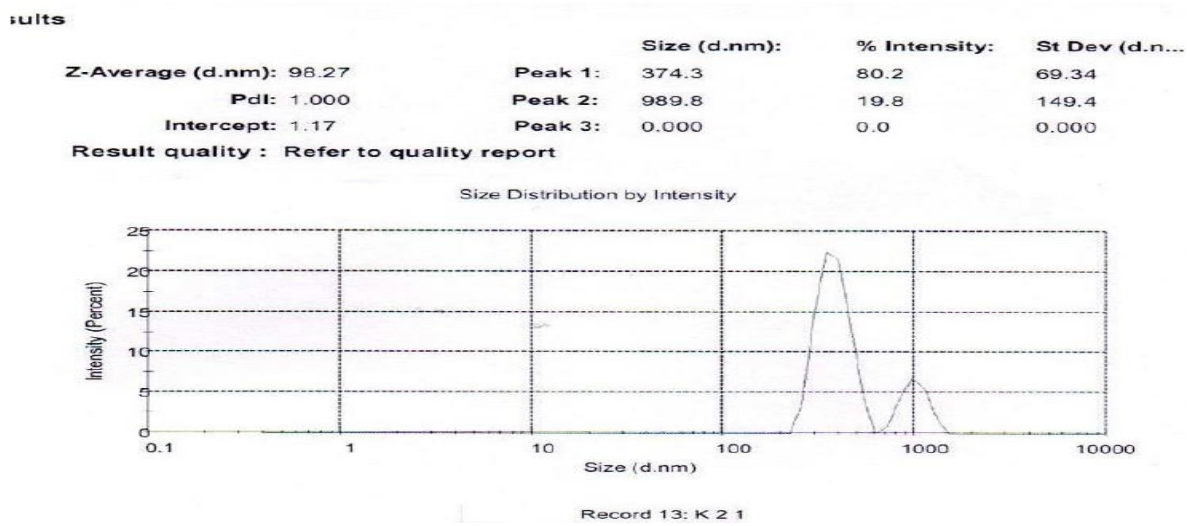

Figure 12: PDI of MTX loaded SLNs.

6.1.2.2 Drug EE and LC

The entrapment efficiency (%) and drug loading (%) was found to be 71.21 and 8.22 respectively.

6.1.2.3 *In vitro* Drug release

The in vitro release profile of the MTX loaded SLNs were investigated by using dialysis beg method. In vitro drug release studies from plain MTX drug solution and MTX loaded SLNs is shown in table.

Table no 10: *In vitro* release data for plain MTX drug solution and MTX loaded SLNs by using dialysis method.

Time (hr)	Plain drug solution (% drug release)	MTX loaded SLNs (% drug release)
0	0	0
1	42.9	16.32
2	59.8	21.5
3	76.48	25.96
4	96.4	32.76
8		38.53
12		42.63
24		56.89

The plain drug solution released more than 40% drug in 1 hr and within 4 hr nearly 100% of drug was released. Whereas, from drug loaded SLNs 25.96% of drug released in 4 hr, followed by 56.89% in 24hr. The release of drug from MTX loaded SLNs experienced an initial brust drug release which followed by constant and continuous release. The initial burst drug release is due to desorption and diffusion of drug from the surface of the SLNs.

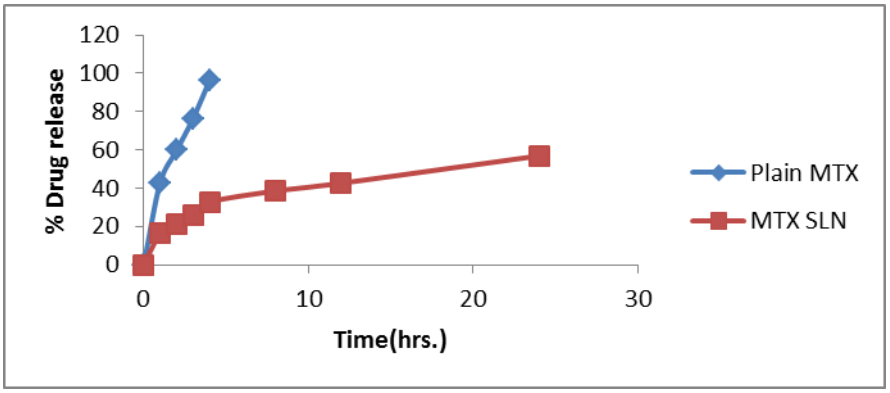

Figure 13: Drug release profile of Plain MTX and MTX Loaded SLNs.

6.1.2.4 Pharmacokinetics study

Pharmacokinetic study which was carried out in Swiss albino mice. Plasmatic profiles of MTX after intraperitoneally administration of plain MTX solution and MTX loaded SLNs are given. The MTX was detected in serum, the maximum concentration of 65.06µg/ml at 6th hr when administered the plain MTX solution but MTX loaded SLNs extended its maximum concentration of 48.04 µg/ml at 10th hr. Therefore, half life of MTX loaded SLNs 14.46 hr that is extended from 8.2 hr which is the half life of plain MTX solution. The extent of absorption of MTX loaded SLNs was higher than that of plain MTX. After MTX loaded SLNs injected by intraperitoneally, the MRT of the drug was found to 23.93 which were higher than plain MTX solution in which the MRT of the drug was 16.07hr.

Table no 11: Pharmacokinetics parameters of plain MTX and MTX loaded SLNs.

S.no.	Parameters	Plain MTX	MTX loaded SLNs
1	Cmax(µg/ml)	65.06	48.04
2	T½	8.2	14.46
3	$[AUC]_o$	1185.37	1997.88
4	$[AUMC]_o$	19051.99	47573.77
5	MRT (hr)	16.07	23.93

6.1.2.5 Biodistribution Study

The biodistribution of MTX after IV administration of plain MTX solution and MTX loaded SLNs. It can be observed that the drug is distributed mainly in the liver, where it is metabolized, and in the kidney which are responsible for elimination. Biodistribution studies were studied for the investigation of the biodistribution of drug in body organ and protein binding properties. Pure drug of MTX having high degree of protein binding. By the formulated MTX loaded SLNs it was reduced. Therefore, report suggested that the biodistribution pattern were modified.

Table no 12: Biodistribution study in different group.

Groups	Brain (mg)	Lungs (mg)	Liver (mg)	Kidney (mg)	Heart (mg)	Blood (mg)
Plain MTX	2.53 ±0.38	7.56 ±0.65	3.54±0.54	3.36±0.06	3.64±0.54	4.92±0.54
Formulation	1.45 ±0.69	4.87± 0.739	2.86±0.650	3.64±0.55	3.88±0.64	2.64±0.75

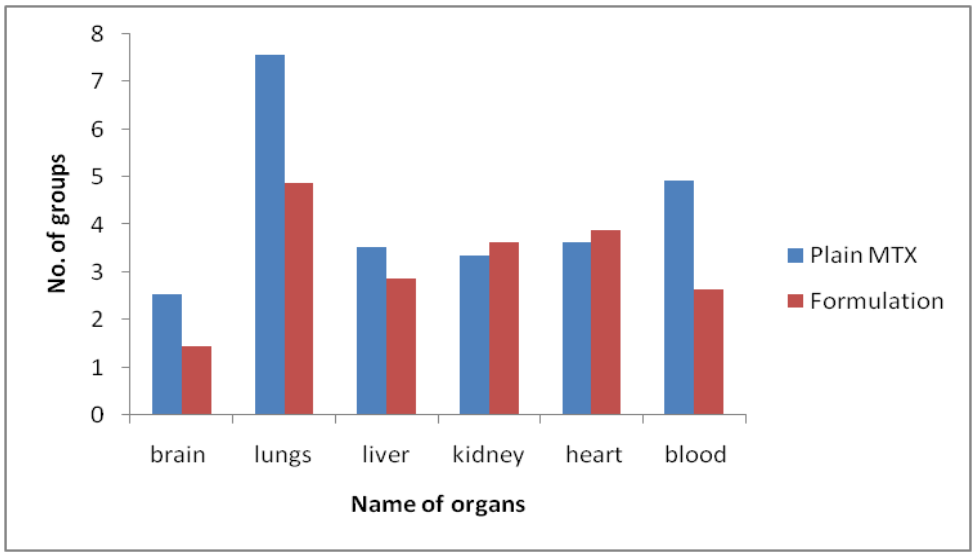

Figure 14: Biodistribution in different group.

DISCUSSION

Toxicity produced with treatment of anticancer drug is vast problem in ancient to till behalf of these many researchers try to establish various approaches to reduced the toxic effect of anticancer drug. So hypothesis suggested the SLNs have vast potential in the field of medical, therapeutic applications as well as diagnostic purpose to improve the quality performance of many products. Methotraxate is one of the antimetabolite agents of the antifolate type used as anticancer drug that have various type of toxicity produced on chemotherapy session. These work using for reduced toxicity and change biodistribution patron of methotraxate. SLN prepared by lipids which are inert in biochemical environment and help to increases the site specific and reduce toxicity. A lipid increases the site specific that promote the change the biodistribution patron directly. There is many type lipids, fatty acids are present which help out to formulate the SLNs. Glyceryl monosterate is one of the biodegradable polymers. It is quite safe for human application and it is very significant to know about the biocompatibility and metabolism of these SLNs. After the formulation of SLNs it's necessary to characterized and evaluated the biodistribution and pharmacokinetics of SLNs for it's in vivo and in vitro stability o formulation.

On this work the characterization parameter of SLNs had been found the morphology size of the particles found to be 120nm that indicate good for Nanoconstruct.

In SLNs the entrapment efficiency and loading capacity was important parameter for the stability of formulation the entrapment efficiency was found to be 71.21% and 8.22%

respectively. After the characterization of SLNs drug release parameter had been studied for invitro release studies of formulation. The in vitro release profile of the MTX loaded SLNs were investigated by using dialysis begs method. The plain drug solution released more than 40% drug in 1 hr and within 4 hr nearly 100% of drug was released. Whereas, from drug loaded SLNs 25.96% of drug released in 4 hr, followed by 56.89 % in 24hr. The release of drug from MTX loaded SLNs exprienced an initial brust drug release which followed by constant and continuous release. The initial burst drug release is due to desorption and diffusion of drug from the surface of the SLNs. After the in vitro studies of the SLNs pharmacokinetics and biodistribution parameter had been studies for the in vivo studies of the formulation. The MTX was detected in serum, the maximum concentration of 65.06µg/ml at 6^{th} hr when administered the plain MTX solution but MTX loaded SLNs extended its maximum concentration of 48.04 µg/ml at 10^{th} hr. Therefore, half life of MTX loaded SLNs 14.46 hr that is extended from 8.2 hr which is the half life of plain MTX solution. The extent of absorption of MTX loaded SLNs was higher than that of plain MTX. After MTX loaded SLNs injected by intraperitoneally, the MRT of the drug was found to 23.93 which was higher than plainMTX solution in which the MRT of the drug was 16.07hr.

The biodistribution of MTX after IV administration of plain MTX solution and MTX loaded SLNs. It can be observed that the drug is distributed mainly in the liver, where it is metabolized, and in the kidney which are responsible for elimination. Biodistribution studies were studied for the investigation of the biodistribution of drug in body organ and protein binding properties. Pure drug of MTX having high degree of protein binding. By the formulated MTX loaded SLNs it was reduced. Therefore, report suggested that the biodistribution pattern were modified.

SUMMARY AND CONCLUSION

It was concluded that, the MTX loaded SLNs were prepared by emulsification solvent evaporation method for the delivery of anticancer agents to human breast cancer cells. It has been observed that SLN has encapsulated considerable amount of MTX and showed smaller particles size and shape, which is suitable for IV administration. The in vitro release profile of MTX from SLNs was carried out to evaluate the stability and release characterization which shows that release from SLNs is dependent on the diffusion of drug through lipid matrix an in vivo degradation of lipid matrix which indicated the diffusion velocity of the

drug to the SLNS surface is very slow, thus, sustained release behaviour is obtained. The pharmacokinetics study proves that its prolonged action.

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