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FORMULATION AND EVALUATION OF VESICULAR DRUG DELIVERY SYSTEM OF SOLUBLE PACLITAXEL COMPLEX

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Abstract

A number of novel vesicular drug delivery systems have been emerged encompassing various routes of administration, to achieve targeted and controlled drug deliveryi.Targeted drug delivery is a mode of delivering the therapeutic agent to the tissues of interest while reducing the relative concentration of therapeutic agent in remaining tissues which improves the therapeutic efficacy and reduces the side effects. **MATERIALS AND METHODS** Liposome was prepared with the help of PTX-HA, Phosphatidylcholine, Cholesterol, Tween 80, PBS, PEG-400. Different process parameter like RPM, Temperature, Time was observed during experiment. PTX-HA Loaded Liposome was prepared. The hepatoprotective activity of pure PTX and PTX-HA complex was performed in Wistar albino rats.**RESULT AND DISCUSSION** Due to poor solubility of PTX, there was low bioavailability and inappropriate tissue localization of PTX that could not show prominent hepatoprotective activity on CCl4 administration. Histopathological study also confirmed a slight diffused liver but with prominent area of hepatocytic cell regeneration. The HA-treated group showed elevated levels of SGOT, SGPT, and ALP (347.67±13.17 U/l, 96.93±8.586 U/l, and 457.06± 16.7 U/l, respectively) same as a toxic group. This indicated that HA did not contribute to hepatoprotective effect as PTX, and at the same time, it did not cause any increase in toxicity. This finding establishes the safety of HA towards liver and promotes it as a valuable pharmaceutical material Significant decrease was observed in serum parameters upon administration of PTX–HA complex when compared to toxic group. **CONCLUSION** It suggested that the complexed form of PTX demonstrated better hepatoprotective activity as compared to free PTX. This might be due to higher solubility of PTX in the complexed form, which caused complete availability for the hepatoprotective action.

KEYWORDS : Vesicular drug delivery system, solubility, paclitaxel complex.

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INTRODUCTION

Novel vesicular drug delivery systems aim to deliver the drug at a rate directed by need of body during the period of treatment, and channel the active entity to the site of action. Biologic origin of these vesicles was first reported in 1965 by Bingham and has been given the name Bingham bodies.

Encapsulation of drugs into liposomes alters the pharmacokinetics and bio distribution, resulting in an increased therapeutic efficacy and decreased toxicity. Despite its strong antitumor activity, paclitaxel (Taxol) has limited clinical applications due to its low aqueous solubility and hypersensitivity caused by Cremophor EL and ethanol which is the vehicle used in the current commercial product. In an attempt to develop a pharmaceutically acceptable formulation that could replace Taxol , a paclitaxel incorporated liposome has been constructed to improve solubility and physicochemical stability.

A number of novel vesicular drug delivery systems have been emerged encompassing various routes of administration, to achieve targeted and controlled drug deliveryii.Targeted drug delivery is a mode of delivering the therapeutic agent to the tissues of interest while reducing the relative concentration of therapeutic agent in remaining tissues which improves the therapeutic efficacy and reduces the side effects.

Drug targeting means the delivery of drugs to receptor, organs or any other specific part of body to which one wishes to deliver the entire drugiii. The targeted drug delivery system was developed by Paul Ehrlich, in 1909, which delivered the therapeutic agent directly to diseased cells. Since then, numbers of carriers were utilized to deliver the drug at target site; these include immunoglobulins, serum proteins, synthetic polymers, microspheres, liposomes, niosomes, erythrocytes etc. Among different carriers, vesicular drug delivery systems are found to be well renownediv. These systems have also been used to improve the therapeutic index, solubility, stability and rapid degradation of drug moleculesv. This article mainly works on formulation and evaluation of vesicular drug delivery system of soluble paclitaxel complex.

MATERIALS AND METHODS

A. LIPOSOME COMPOSITION

Liposome was prepared with the help of following ingredients shown in Table no.1

Table No.1: - Liposome composition comprises of following ingredients

Ingredient	**B1**	**B2**	**B3**
PTX-HA	30mg	30mg	30mg
Phosphatidylcholine	0.5gm	1.0gm	1.5gm
Cholesterol	0.5gm	1.0gm	1.5gm
Tween 80	0.5gm	1gm	1.5gm
PBS	4	4	4
PEG400	0.5%	1%	1.5%

The different process parameter was observed during experiment shown in Table no. 2

Table No.2:- Process Parameter was observed during experiment

Process Parameter	**B1**	**B2**	**B3**
RPM (per minute)	5000	10000	15000
Temperature (Celsius)	30	40	50
Time (Min)	30	40	50

B. PTX-HA Loaded Liposome Preparation Method

PTX – HA loaded liposome preparation method is as follows

- PTX-HA, Lipid and cholesterol dissolved in chloroform
- Dried in a rotary evaporator and water bath with temperature 40°C
- The thin film layer is formed
- The thin film layer was suspended in PBS 4 containing Tween 80 and PEG 400 by rotating the flask at 300 rpm till lipid film is completely hydrated
- Then liposome dispersion was serially passed through 1.2,0.4 and finally 0.2 µm pore size filled.
- Untrapped PTX was removed from liposome dispersion by centrifuging at 10,000 rpm for 30 min. Supernatant was discarded and liposomes washed with two times with PBS 4
- Final liposomes powder were stored in tight container at 4°C for further studies

C. EVALUATION OF OPTIMIZED BATCH

Evaluation of optimized batch with the help of different Techniques was shown in Table no.3

Table No.3: - Characteristics with representative Technique

Characteristics	**Representative Technique**
Particle size and size distribution	Electron microscopy
Morphology	Electron microscopy
Surface charge	Zeta potential analysis
Encapsulation efficiency	drug analysis (HPLC)
Release rate	Release in physiological media or storage buffer
Physical stability	Particle size change in physiological media or storage buffer

D. Assessment of hepatoprotective activity

The hepatoprotective activity of pure PTX and PTX-HA complex was performed in Wistar albino rats. The experimental procedure and protocol were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Modern College of Pharmacy, Nigdi, Pune, India, constituted under Committee for purpose of control and Supervision of Experiments on

Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Approval no. MCP/IAEC/49/2022). Ethical guidelines were strictly followed during all the experiments. Wistar Albino rats (150– 200 g) obtained from the animal house of Modern College of Pharmacy, Nigdi, Pune, India. The method for hepatoprotective activity is as follows

- Standard laboratory conditions of temperature 24 ± 2 C, relative humidity 55 ± 5%, and 12:12 h light dark cycle were maintained throughout all the experiments. Total 25 animals were used. Animals were divided into five groups, each of five rats. Group 1 was kept as a control group and received only vehicle (distilled water) via the oral route. Group 2 acted as toxic control and received distilled water for seven consecutive days. Group 3 receive aqueous HA solution equivalent to that present in the complex, Groups 4 received a six-day repeated oral dose 20 mg/kg PTX suspended in aqueous solution of 0.5% HPMC K15.
- Group 5 received PTX-HA complex equivalent to 20 mg/kg of PTX. At the end of six days, all the groups except Group 1 were administered a 2 ml/kg single oral dose of CCl4. On the seventh day, the blood was collected from the retro orbital plexus of each animal under ether anesthesia. The samples were centrifuged at 7000 rpm for 10 min within one hour after collection to separate serum.
- Collected serum was biochemically tested for transaminase levels of both types i.e. serum glutamic–oxaloacetic transaminase (SGOT) and serum glutamic–pyruvate transaminase (SGPT) as well as serumalkaline phosphatase (SALP) level by an autoanalyzer (Erba Chem Touch, Mannheim, Germany). After collecting the blood from each animal, animals were sacrificed. Liver was immediately separated, fixed in 10% formalin, serially sectioned and microscopically examined under a microscope (Motic, DMB1 Digital Biological Microscope, Xiamen, China) at 40 after staining with hematoxylin and eosin to analyze any pathological changes.

RESULTS AND DISCUSSION

1. Study of Physicochemical properties of Polymer

Study of Computed properties was shown in table no.4

Table No:-4 Study of Computed properties

Parameter	Values
Molecular Weight	853.9
XLogP3	2.5
Hydrogen Bond Donor Count	4
Hydrogen Bond Acceptor Count	14
Rotatable Bond Count	14
Exact Mass	853.33095530
Monoisotopic Mass	853.33095530
Topological Polar Surface Area	221 Å²
Heavy Atom Count	62
Formal Charge	0
Complexity	1790
Isotope Atom Count	0
Defined Atom Stereocenter Count	11
Undefined Bond Stereocenter Count	0
Covalently-Bonded Unit Count	1
Compound Is Canonicalized	Yes

Study of physicochemical properties of polymer was shown in Table no.5

Table No.5:- Physicochemical Properties of Polymer

Parameter	Values
Color/Form	White to off-white crystalline powder
Melting Point	216-217 °C
Solubility	Insoluble in water
LogP	3
Stability/Shelf Life	Bulk samples stored at room temperature for 30 days showed no TLC or HPLC decomposition as indicated by UV absorption
Optical Rotation	-49 deg at 20 °C/D (methanol); UV max absorption

2. Physicochemical Properties of Liposomes

The physical properties like vesicle shape, vesicle size, spreadability and the chemical properties like pH of liposomes were performed and the results are presented in the Table. The vesicle size of all four batches was in the range and the shape was perfectly spherical as shown into microscopical image of liposomes in figure. The spreadability found was good with pH ranging from 6.81 to 6.86 holding them most acceptable to avoid the risk of irritation after application on the skin was shown in fig no.1.

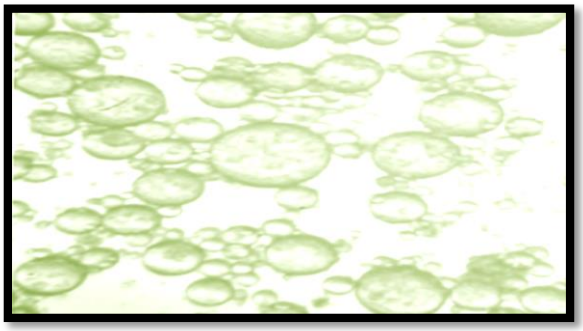


Fig no.1.: - Microscopical study of formulated batches

3. Characterization Liposomal Batches

Characterization of liposomal batches are shown in table no.6

Table no.6: - Characterization of liposomal batches

Batches	Vesicle Size(nm)	Vesicle Shape	Spreadability* (gm.cm/sec)	pH*	Viscosity (cp)
B1	240 - 760	Spherical	39.1 ± 0.28	6.86 ± 0.02	193.56
B2	**140 - 517**	**Spherical**	**57.3 ± 0.36**	**6.81 ± 0.01**	**112.83**
B3	160 - 620	Spherical	44.9 ± 0.31	6.83 ± 0.04	96.23
B4	310 - 840	Oval	36.8 ± 0.26	6.84 ± 0.02	268.29

*** SD: Standard deviation, (n=3).**

4. **Drug Loading Capacity**

The drug loading capacity was same as the entrapment efficiency which gives the idea of amount of drug loaded per unit weight of the vesicle. It was an important parameter for the nano medicines. B2 had maximum loading capacity. Loading capacity of B4 was minimum. The other two batches had almost same drug loading capacity.

5. **Entrapment efficiency:**

The entrapment efficiency (EE) ranges from 42.2 ± 1% to 88.5 ± 2%. B2 batch had the maximum whereas B4 batch had the lowest %EE. The maximum %EE of B2 batch was due to the average more amounts of the phospholipids, polyglycol shown in fig no.6

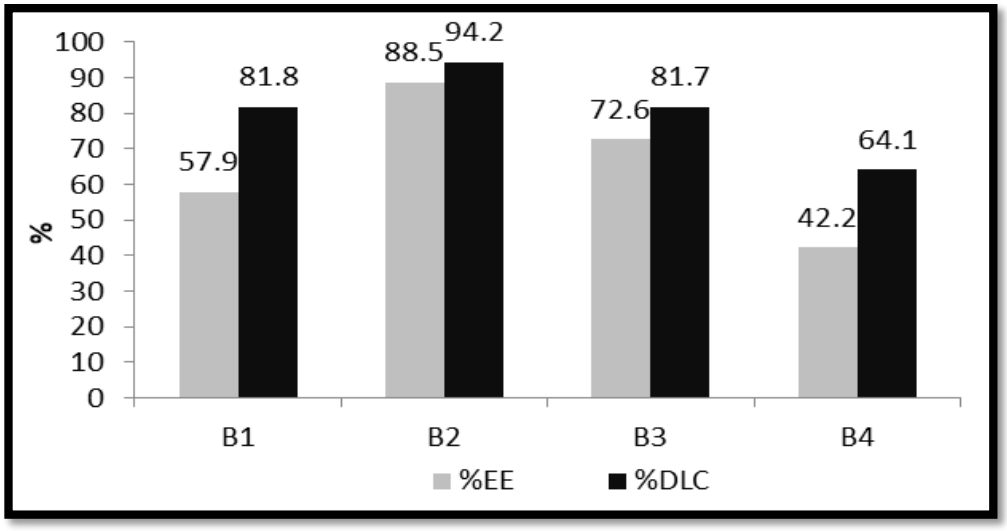
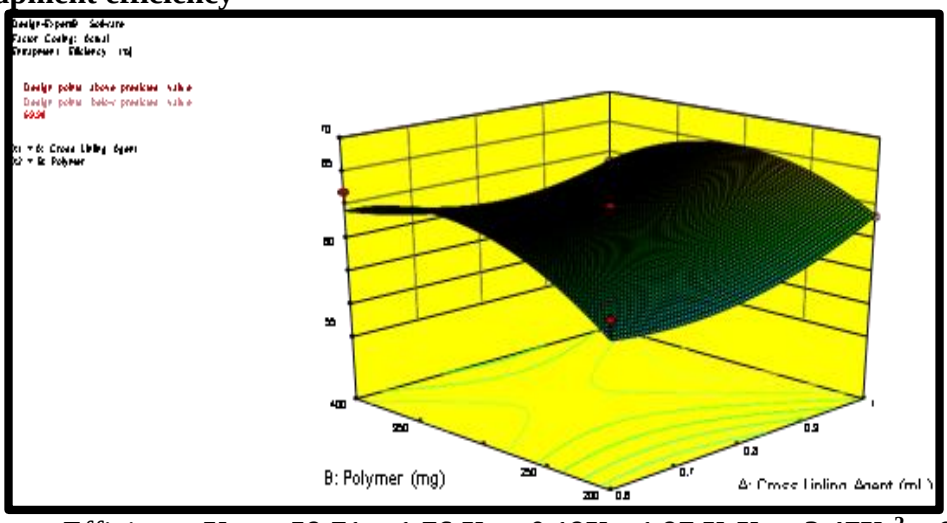


Fig no.2: -Comparative study of %DLC and %EE of Liposomal formulation

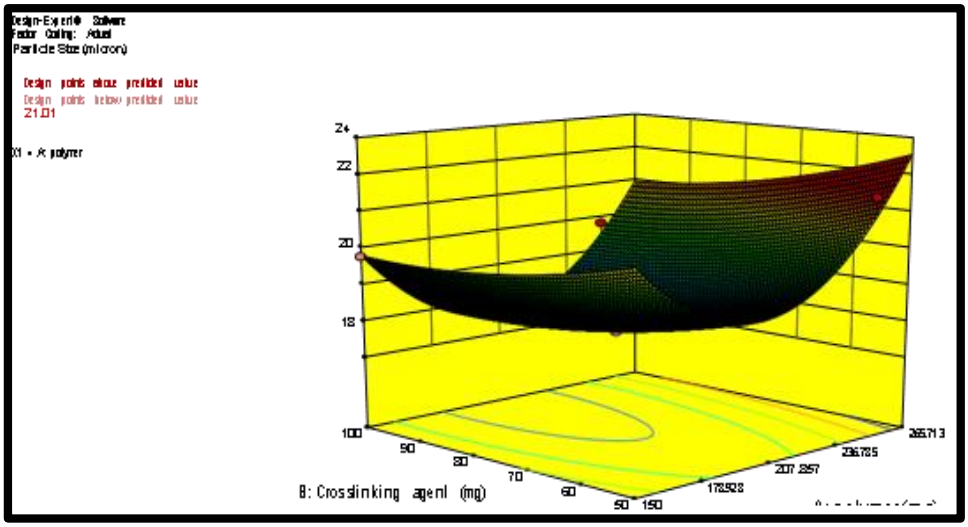
6. **Response surface plots of PTX-HA Liposomal Formulation**

A) Entrapment efficiency



Entrapment Efficiency $\mathbf{Y_1 = +59.71 + 1.76\ X_1 + 0.19X_2 - 1.97\ X_1X_2 + 3.47X_1^2 - 6.36\ X_2^2}$

B) Entrapment efficiency



7. ANOVA results of measured responses

ANOVA results of measured responses was shown in table no. 7

Table No.7: - ANOVA results of measured responses

ANOVA Results for measured responses (Y)			
		ARTL	
ANOVA	Coefficient	Y1	**Y2**
	F Value	9.55	**2.52**
	P Value(Prob>F)	**0.0462**	**0.0029**

Values of "Prob> F" less than 0.0500 indicate model terms are significant

8. ZETA POTENTIAL

Zeta potential provides a measurement of the net charge on the liposomal surface. It was determined using zeta potential analyzer (nanoparticle analyzer Horiba SZ-100) at 25.1°C. The measurement of Zeta potential gives electrophoretic mobility and means zeta potential values were obtained directly from the measurement. The Zeta potential of B2 Formulation was shown in fig no.3

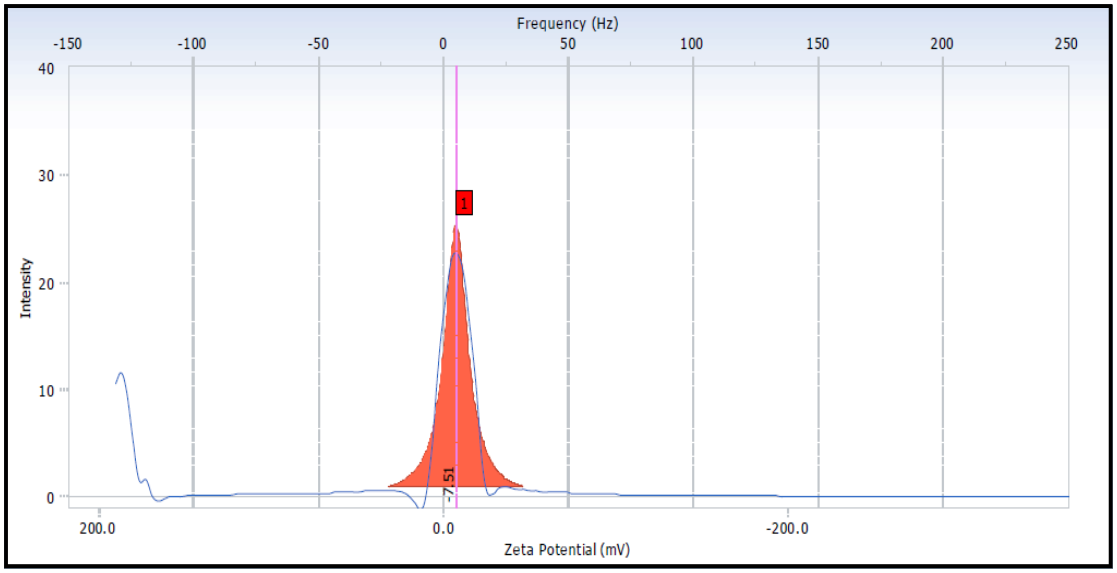


Fig no.3 : Zeta Potential of B2 Formulation

The significance of zeta potential is that its value can be co- related to the stability of colloidal dispersions. Zeta potential value higher than ± 30 mv show good physical stability, being optimized when they reach approximately -60mv, exhibiting a very good physical stability during the shelf-8 life. The value of zeta potential was found to be -7.51 mv.

9. In-Vitro Diffusion Studies

The In- Vitro Diffusion studies results was shown in Table no.

Table no.8: - The In- Vitro Diffusion studies results

TIME (min)	%CDR(Cumulative drug release)			
	B1	**B2**	**B3**	**B4**
0	0.00	**0.00**	0.00	0.00
60	8.80	**9.28**	8.25	7.21
120	16.48	**18.64**	15.71	13.33
180	27.08	**32.01**	28.73	23.56
240	40.10	**45.52**	40.02	36.18
300	54.04	**62.52**	53.48	48.31
360	66.40	**75.64**	70.02	57.94
420	72.02	**85.38**	77.91	63.12

10. **In-vitro drug diffusion studies Formulations**

In-vitro drug diffusion studies Formulations graph was shown in fig no.4

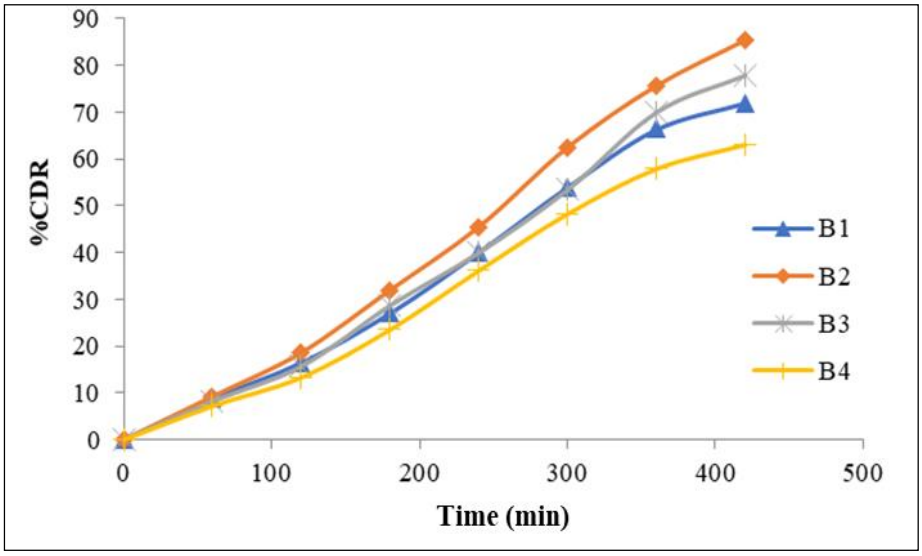


Fig no.4: - In-vitro drug diffusion studies Formulations

11. In-vitro drug diffusion studies among the four batches of PA-HX liposome Batches

Organoleptic Characterization and Physicochemical Evaluation of Liposomal Batch was shown in Table no.9

Table No.9: - Organoleptic Characterization and Physicochemical Evaluation of Liposomal Batch

Batches	**Texture**	**Homogeneity**	**Consistency**	**Viscosity (cps) (mean ± SD*)**	**pH (mean ± SD*)**	**Spreability (gm.cm/sec)**	**Tube extrudability (g/cm^2) (mean ± SD*)**
B1	Smooth	Excellent	Excellent	48 251.3 ± 5.12	6.73 ± 0.05	22.68 ± 0.91	27.21 ± 0.89
B2	**Smooth**	**Excellent**	**Excellent**	**38 085.1 ± 4.34**	**6.66 ± 0.01**	**32.34 ± 1.66**	**39.14 ± 1.53**
B3	Smooth	Excellent	Excellent	43 163.6 ± 5.41	6.53 ± 0.04	27.10 ± 1.34	33.16 ± 1.41
B4	Smooth	Good	Fair	56 289.4 ± 7.64	6.76 ± 0.02	16.49 ± 0.72	21.91 ± 0.85

12. ***In-vitro* Drug Release and Kinetic Study:**

Given table no. 10 shows that the B2 had maximum drug release than all the other batches. The drug release of PTX-HA Liposome ranges from 59.85 % - 81.06 %.

Table no 10.: - The drug kinetic Time Vs % CDR

TIME(min)	**%CDR**			
	B1	**B2**	**B3**	**B4**
0	0.00	**0.00**	0.00	0.00
15	8.62	**8.95**	7.86	6.45
30	14.06	**14.09**	14.26	11.91
60	18.35	**24.15**	26.61	21.62
120	26.92	**37.65**	38.13	33.16
210	38.56	**59.83**	50.37	44.17
330	51.38	**73.39**	66.51	52.44
480	64.22	**81.06**	73.15	59.86
Kinetic Model	**R^2**			
	B1	**B2**	**B3**	**B4**
Zero Order Kinetic	0.96	**0.97**	0.98	0.99
First Order Kinetic	0.87	**0.90**	0.94	0.97
Higuchi's Model	0.82	**0.82**	0.85	0.86
Peppa's Model	0.96	**0.96**	0.98	0.98
Best Fitted Model	***Zero Order***	***Zero Order***	***Zero Order***	***Zero Order***

%CDR: % Cumulative drug release

13. **Stability Study:**

The change in the results of stability study was negligible. The fractional change in the viscosity indicated that the gel formulation was physically stable till 90 days. The pH remained unchanged which indicated it is chemically stable and non irritants to the skin. The drug content remained same and also the drug release didn't change. All the formulation gave good results so the liposomes prepared were stable till the end of 90 days. It was given in Table.11

Table no.11: - Stability of study of formulations

Parameters	**Batches**	**Initial Day**	**After 30 Days**	**After 90 Days**
Viscosity (cps)	B1	48.251.3 ± 5.12	48 404.1 ± 6.23	48.889.5 ± 5.46
	B2	38.085.1 ± 4.34	38 245.3 ± 5.38	38.605.9 ± 4.35
	B3	43.163.6 ± 5.41	43 354.1 ± 5.69	43.736.6 ± 6.98

	B4	56.289.4 ± 7.64	56 499.4 ± 8.03	56.846.1 ± 8.45
pH	B1	6.73 ± 0.05	6.70 ± 0.05	6.64 ± 0.06
	B2	6.66 ± 0.01	6.61 ± 0.06	6.57 ± 0.02
	B3	6.53 ± 0.04	6.49 ± 0.03	6.42 ± 0.03
	B4	6.76 ± 0.02	6.71 ± 0.01	6.62 ± 0.02
% Entrapment Efficacy	B1	57.9 ± 1.05	55.1 ± 1.56	51.3 ± 1.68
	B2	88.5 ± 2.14	86.6 ± 7.14	84.9 ± 6.03
	B3	72.6 ± 2.04	70.7 ± 1.86	64.6 ± 7.42
	B4	42.2 ± 1.85	39.5 ± 7.04	33.4 ± 8.06
% Drug Content	B1	55.1 ± 1.08	98.19 ± 3.48	97.85 ± 3.79
	B2	51.3 ± 1.02	98.89 ± 1.97	98.01 ± 2.16
	B3	98.93 ± 2.04	98.13 ± 3.78	97.23 ± 3.98
	B4	99.39 ± 3.16	98.66 ± 3.37	97.49 ± 4.79
% Drug released (At 420 min)	B1	64.22	63.65	60.59
	B2	81.06	79.39	77.95
	B3	73.15	69.97	66.87
	B4	59.85	56.89	53.63

14. **Particle Size Distribution**

The PTX-HA liposomal formulation particle size of B2 batch was found to be 113.34µm ± 0.97 with the polydispersity index of 13.41± 0.14.

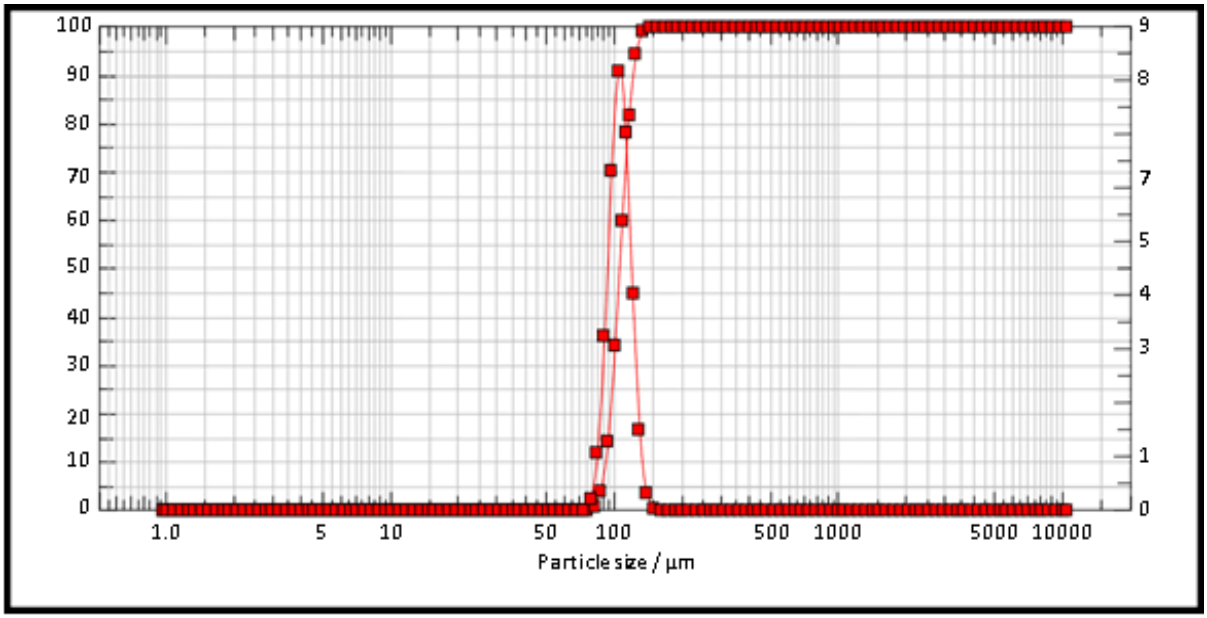


Fig no. 5: - Particle Size Distribution

15. **Particle size of PTX-HA Formulation (B2)**

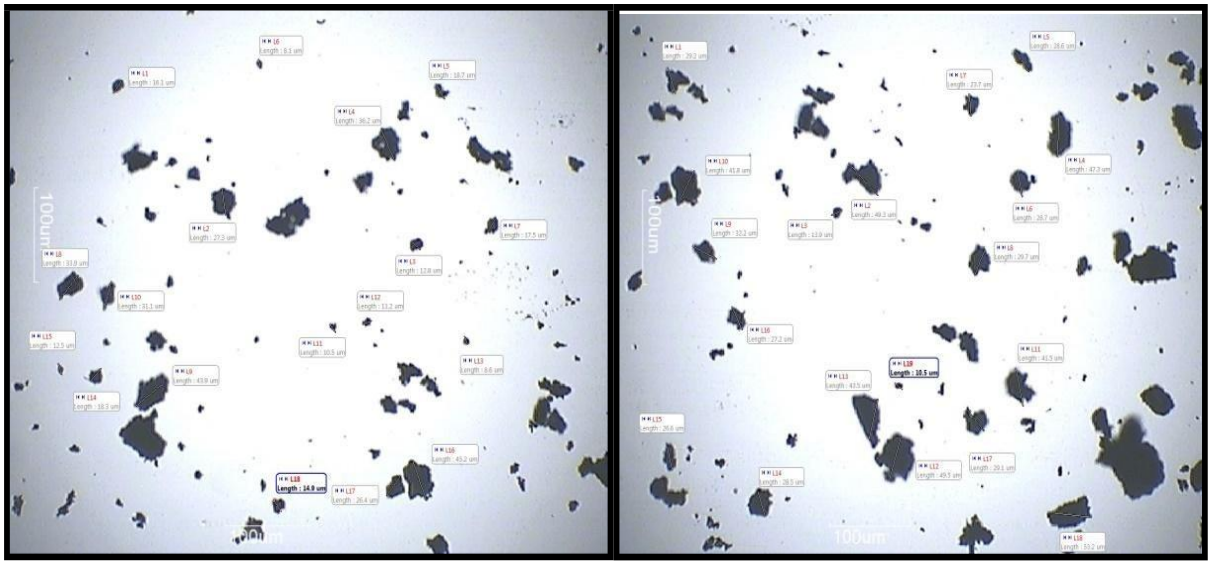


Fig no.6: - Photomicrographs of A)PTX-HA Liposomal Formulation (50X) B) A)PTX-HA Liposomal Formulation (60X) shows particle size distribution

16. Hepatoprotective Activity

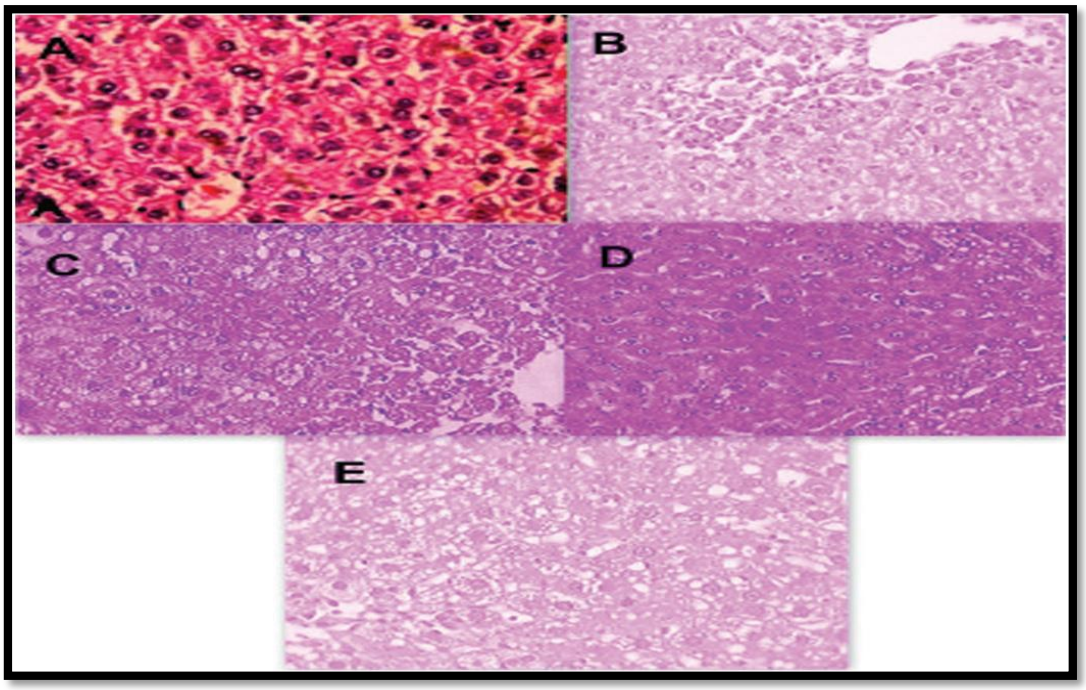


Fig no. 7: -Representative photographs of liver sections of albino rats: (A) normal control, (B) toxic control, (C) treated with PTX, (D) treated with HA, and (E) treated with HA–PTX complex as observed after staining with hematoxylin and eosin under Motic digital microscope (40).

Table no. 12: - Hepatoprotective evaluation of PTX against CCl4-induced hepatotoxicity in rats compared with toxic control group.

Sr.No.	Group	SGOT±SEM (U/l)	SGPT±SEM (U/l)	ALP±SEM (U/l)
1	**NC**	203.50 ± 0.50**	53.7 5 ± 0.75**	175.80 ± 0.60**
2	**TC**	358.80 ± 32.69	96.50 ± 3.00	472.75 ± 26.75
3	**PA**	342.07 ± 21.12 ns	129.30 ± 5.30*	336.60 ± 30.40**
4	**HA**	347.67 ±13.17 ns	96.93 ± 8.58 ns	457.06 ±16.70 ns
5	**CA**	244.33 ±10.13**	61.90 ± 7.49**	290.74 ±11.14**

SGOT= serum glutamic–oxaloacetic transaminase;

SGPT= serum glutamic–pyruvate transaminase; ALP, serum alkaline phosphatase;

NC,normal control;

TC, toxic control;

PA, pure drug;

HA, humic acid;

CA, HA–PTX complex;

*p < 0.01

*** p < 0.05**

Due to poor solubility of PTX, there was low bioavailability and inappropriate tissue localization of PTX that could not show prominent hepatoprotective activity on CCl4 administration. Histopathological study also confirmed a slight diffused liver but with prominent area of hepatocytic cell regeneration. The HA-treated group showed elevated levels of SGOT, SGPT, and ALP (347.67±13.17 U/l, 96.93±8.586 U/l, and 457.06± 16.7 U/l, respectively) same as a toxic group. This indicated that HA did not contribute to hepatoprotective effect as PTX, and at the same time, it did not cause any increase in toxicity. This finding establishes the safety of HA towards liver and promotes it as a valuable pharmaceutical material Significant decrease was observed in serum parameters upon administration of PTX–HA complex when compared to toxic group. It shows SGOT, SGPT, and ALP (244.33 ± 10.13 U/l, 61.9 ± 7.49 U/l, and 290.74 ± 11.14 U/l, respectively) levels which are comparable to the normal range. It suggested that the complexed form of PTX demonstrated better hepatoprotective activity as compared to free PTX. This might be due to higher solubility of PTX in the complexed form, which caused complete availability for the hepatoprotective action. The complex-treated group showed normal liver cell texture with prominent hepatocytic regeneration activity around necrotic areas. It also indicated minimal infiltration by lymphocytes in portal tracts decrease in SGOT and SGPT towards normal value indicated stabilization of plasma membrane and repair of hepatic tissue damage while decrease in ALP value up to normal suggested the stability of biliary function.

CONCLUSION

Encapsulation of drugs into liposomes is a versatile and effective drug delivery strategy that has been used for several decades. Liposomes offer numerous advantages over traditional drug delivery methods, including enhanced bioavailability, targeted delivery, and sustained drug release. Advances in liposome technology continue to expand the range of drugs that can be encapsulated and the properties of liposomes that can be engineered, making them an important tool in the development of new therapies. The vesicle size of all four batches was in the range and the shape was perfectly spherical as shown into microscopical image of liposomes B2 had maximum loading capacity. Loading capacity of B4 was minimum. The other two batches had almost same drug loading capacity. The entrapment efficiency (EE) ranges from 42.2 ± 1% to 88.5 ± 2%. B2 batch had the maximum whereas B4 batch had the lowest %EE. The maximum %EE of B2 batch was due to the average more amounts of the phospholipids, polyglycol. Zeta potential provides a measurement of the net charge on the liposomal surface. It was determined using zeta potential analyzer (nanoparticle analyzer Horiba SZ-100) at 25.1°C. The measurement of Zeta potential gives electrophoretic mobility and means zeta potential values were obtained directly from the measurement. Due to poor solubility of PTX, there was low bioavailability and inappropriate tissue localization of PTX that could not show prominent hepatoprotective activity on CCl4 administration. Histopathological study also confirmed a slight diffused liver but with prominent area of hepatocytic cell regeneration. It suggested that the complexed form of PTX demonstrated better hepatoprotective activity as compared to free PTX. This might be due to higher solubility of PTX in the complexed form, which caused complete availability for the hepatoprotective action.

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