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Formulation design and evaluation of xyloglucan microsphere of silymarin

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The objective of the present work was to prepare and systematically evaluate *in vitro* and *in vivo* performances of xyloglucan microsphere of silymarin. Microspheres were prepared by the emulsification method using glutaraldehyde as a cross-linking agent. Surface morphological characteristics of microspheres investigated using scanning electron microscopy and light microscopy. The drug polymer compatibility, percentage of yield, swelling index, drug entrapment efficiency and in vitro drug release studies were performed and the drug release data was treated with mathematical kinetic models. The pharmacokinetic experiments showed that the area under the curve of silymarin plasma concentration–time profile in rats for standard silymarin was lower than silymarin entrapped xyloglucan microspheres. Tissue distribution studies of xyloglucan microspheres indicated that a significantly increased amount of silymarin was accumulated in the liver. The present study was focused on development of silymarin microspheres by using biodegradable polymers and to study the effect of method of preparation on physical properties, *in vitro* drug release profile and *in vivo* study of xyloglucan microspheres.

Keyword: Tamarind seed Polysaccharides, Xyloglucan Microspheres, Silymarin, Liver target drug delivery

1. Introduction

Natural gums and polysaccharides and their derivatives represent a group of polymers widely used in pharmaceutical dosage forms. Polysaccharides are the choice of materials among the hydrophilic polymers used, because they are nontoxic and acceptable by the regulatory authorities [1].

Tamarind seed polysaccharide (TSP) is a biodegradable polysaccharide extracted from Tamarind seeds (*Tamarandus indica* Linn. Family; *Leguminosae*) called as TSP has been found to have a wide application in pharmaceutical industry [2]. This led to its application as stabilizer, thickener, gelling agent in food and binder in pharmaceutical industries [3].

It is extracted, purified and refined and used as a thickening, stabilizing and gelling agent [4]. Purified TSP is a high-molecular-weight, non-ionic, neutral, branched polysaccharide consisting monomer of D-glucose, D galactose and D-xylose in molar ratio of 3:1:2 [5]. Xyloglucan polysaccharide has a (1-4)-β-D-glucan backbone chain with (1-6)-α-D-xylose branches that are partially substituted by (1-2) - β - D- galactose xylose [6]. It is insoluble in organic solvents and dispersible in hot water to form a highly viscous gel such as a mucilaginous solution with a broad pH tolerance and adhesivity [7, 8]. In addition, it is nontoxic and nonirritant with haemostatic

activity[7]. It had been previously used in some drug formulations [9, 10]. In addition to these other important properties of xyloglucan have been identified recently. They include non-carcinogenicity, mucoadhesivity, biocompatibility, high drug holding capacity and high thermal stability. This led to its application as excipients in hydrophilic drug delivery system [11]. The release kinetics of both water-soluble and water insoluble drugs from the TSP matrix which also showed high thermal stability [12]. It is used as binder in tablets, gelling agent, thickening agent, as emulsifier, release retardant and as stabilizer in food, and pharmaceutical industrie [13].

Targeted drug delivery to the liver is achieved using hepatic asiagloglycoprotein (ASGPRS) receptor mediated endocytosis [14]. Liver associated surface receptors, the asialoglycoprotein receptor (galactose receptor) is known to be present only on the hepatocytes [15], with high density of 5,00,000 receptors per cell (Schwartz *et al*., 1980) [16] and retained on several human hepatoma cell lines [17]. In addition, once a ligand binds to the galactose receptor, the ligand-receptor complex is rapidly internalized and the receptor recycles back to the surface, which would allow the high binding capacity and efficient cellular uptake of galactosylated ligands [18].

It was reported that asialoglycoprotein (ASGP) receptors were expressed plentifully on the surface of hepatocyte cells and targeting could be accomplished through the introduction of galactose residues which can bind specifically to the ASGP receptors on hepatocytes cells, into drug carriers for the treatment of liver disease [19].

Tamarind seed xyloglucan (XG) as a new synthetic extra cellular matrix (ECM) for hepatocyte attachment, they found that hepatocytes formed spheroids mediated by ASGPR when cultured on the polystyrene surface coated with xyloglucan as similar to poly [N-p-vinylbenzyl-4-O-b-D-galactopyranosyl-D-gluconamide] (PVLA) [19]. Xyloglucan having galactose moieties, which specifically interacts with hepatocytes, hepatocyte spheroids maintained high liver-specific functions after ASGPR mediated adhesion onto xyloglucan based on alginate capsules. The results suggest that the multicellular spheroid formation of hepatocytes in the presence of xyloglucan as a new synthetic ECM can enhance the liver-specific functions in the three dimensional space [19, 20].

Silymarin, the seed extract of the milk thistle (*Silybum marianum),* is a unique hepatoprotective agent that has a positive effect on the metabolism and physiology of liver cells [21]. The drug also prevents toxic and foreign substances from penetrating liver cells by stabilizing the outer membranes of these cells [22].

Biodegradable microspheres based on natural polymers are frequently employed as drug delivery systems to achieve a controlled release and site-specific targeting of the incorporated drug. The objective of the present study was to develop formulations (microspheres) based on xyloglucan view to enhancing the liver delivery of silymarin through entrapment into these microspheres.

2 Materials and method

2.1 Materials

Tamarind kernel powder was obtained as a gift sample from Prepem Gums Pvt. Ltd, Mumbai, India. Silymarin drug was obtained as a gift sample from Micro Lab Bangalore, India. Castor oil, glutaraldehyde and span 80 were purchased from CDH (P) Ltd, New Delhi, India. Absolute ethanol was purchased from Merck Ltd, Mumbai, India. All the chemicals used were of A.R grade.

2.2 Method

I. Isolation of tamarind seed polysaccharide

TSP was isolated following the method reported by (Rao, *et al*. 2002) [8]. Briefly, to 20 g of tamarind kernel powder, 200 ml of cold distilled water was added and slurry was prepared. The slurry of TSP was poured into boiling distilled water. The solution was boiled, and then centrifuged at 5000 rpm for

Table 1: Formula for preparation of Silymarin microsphere

S.no	Amount of drug: polymerconcentration	Amount of castor oil (ml)	Concentration of span 80 (%)	Volume of glutaraldehyde (ml)
1	1:2	40	1.25	1.5
2	1:3	40	1.25	1.5
3	1:4	40	1.25	1.5

20 minutes. The supernatant was separated and was precipitated using absolute ethanol. The product was filtered, washed and dried under freeze dryer. (Vertis, wizard 2.0)

II. Preparation of xyloglucan microspheres

Xyloglucan microspheres were prepared by the emulsification method reported by (Chaurasia, at al. 2007) [23] and the hardening of microspheres was performed by chemical cross-linking with glutaraldehyde as well as with temperature-induced cross-linking. An aqueous dispersion of xyloglucan (20 ml) containing 2% w/v of xyloglucan (an accurately weighed amount of gum was dispersed in a specified volume of cold water containing the drug and allowed to swell for 2 hr). This solution was dispersed in 40 ml of castor oil containing 2% of span 80 the mixture was than stirred continuously using mechanical stirrer at 3000 rpm for 30 minutes at room temperature. After complete mixing 1.5 ml of glutaraldehyde were added to the dispersion. The harden microspheres were filtered and washed with acetone to remove castor oil, unrelated glutaraldehyde and span 80. The microspheres were then freeze dried. (Table 1)

2.3 Optimization of process variables

1. Surface morphology

The samples were prepared by lightly sprinkling the microsphere powder on a double side adhesive tape, which already shucked to on aluminum stubs. The stubt were then placed in to ion coat sputter for gold coating. After go coating sample were randomly scanned for shape surface morphology. (Figure 1)

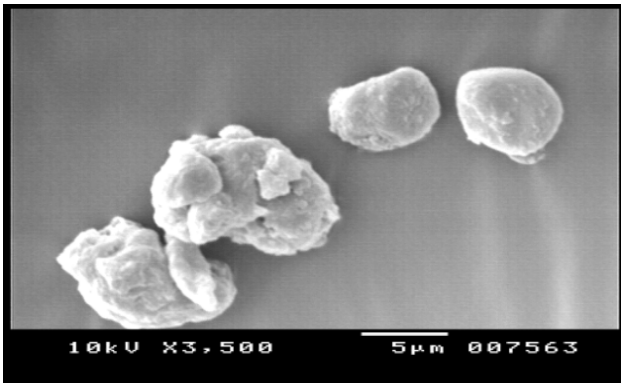

Fig 1: Scanning electron micrographs of xyloglucan microspheres

II. Light microscopy

A small amount (mg) of each sample was suspended in PBS (pH7.4) and was placed onto a mechanical stage of fluorescence microscopy (Radical India) and samples were randomly scanned for shape then photograph was taken. (Figure 2)

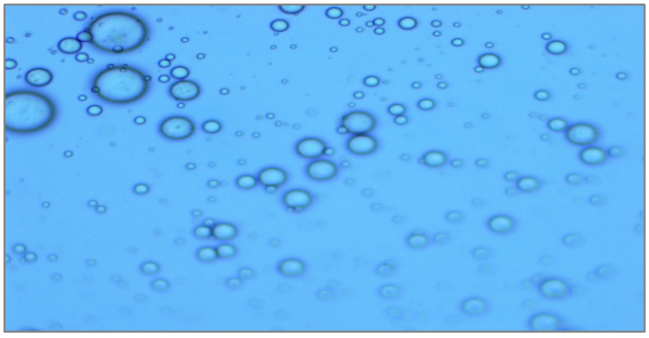

Fig 2: Light microscope photograph of xyloglucan microspheres

III. Drug-polymer interaction study

Drug polymer interaction was carried out by differential scanning calorimetry (Trade Perkins Elmer UK). An accurately weighed amount (2 mg) of the pure silymarin, xyloglucan and blend of silymarin and xyloglucan was place in an aluminum cell heating the sample from 30 ^{0}C to 450 ^{0}C at the heating rate of 10 ^{0}C/min in a nitrogen environment. (Figure 3)

IV. Particle size distribution

The particle size and size distribution were determined by using Zetasizer (Malvern Instrument Ltd UK). The mean diameter and size distribution were determined by Zetasizer Malvern. For analysis the freeze dried microspheres were dispersed in PBS (pH 7.4). The particle size is expressed as volume mean diameter ±S.D of values collected from three different batches.

V. Yield (%) of microspheres formulation

The yield of microspheres obtained after using different variable. The % yield of formulation was determined by formula given below-

$$\text{Percentage yield} = \frac{\text{Total weight of microspheres}}{\text{Total weight of drug + Total weight of polymer}} \times 100$$

……..Eq (1)

VI. Entrapment efficiency

Entrapment efficiency is the amount of added drug (in percent) that is entrapment in the microspheres. Entrapment efficiency was calculated in term of the ratio of drug in the final formulation to the amount of added drug. An accurately weighed amount (100 mg) of the formulation of microspheres was dispersed in 100 ml of PBS (pH 7.4). The sample was ultrasonicated for 3 consecutive periods of 5 minutes each, with a resting period of 5 min each. It was left to equilibrate for 24 hr at room temperature, and the suspension was then centrifuged at 3000 rpm for 15 min. The supernatant was diluted appropriately with PBS (pH 7.4) and analyzed for concentration of silymarin at 288 nm λmax using 1700 UV/Visible spectrophotometer (Shimadzu, Japan).

The entrapment efficiency was determined by formula given below-

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} X100$$

……..Eq (2)

VII. Swelling study

The equilibrium water uptake of the empty and drug loaded microspheres were determined by measuring the extend of swelling in water.

A pre-weighed amount (100 mg) of microspheres was placed in simulated gastric fluid (SGF) (pH 7.4) and allowed to swell up to a constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured.

The swelling index was determined by formula given below:

$$\text{Swelling index} = \frac{\text{Mass of swollen microsphere} - \text{Mass of dry microsphere}}{\text{Mass of dry microspheres}} X100$$

……..Eq (1)

VIII. *In vitro* drug release studies

VIII.I Procedure for in vitro drug release study

The procedure of *in vitro* release study was carried out by using prepared biological membrane. A two ml of the final preparation taken in biological membrane pouch, dipped in 100 ml of phosphate buffer saline (pH 7.4) with continue stirring on a magnetic stirrer and temperature was maintain at 37 $^0C \pm 2^0$. Sampling was done after each hr by withdrawing 1 ml of sample and same amount of fresh media was added. Sample was diluted up to 10 ml using fresh media and absorbance was measured at 288 nm λ_{max} in 1700 UV/Visible spectrophotometer (shimanzu, Japan).

IX. *In-Vivo* study

The purpose of the present study is to examine the tissue distribution and pharmacokinetics of the xyloglucan microspheres after oral administration to rat. The silymarin concentrations in major organs, target site, and plasma were quantitatively analyzed by an HPLC method and pharmacokinetic parameters were calculated based on plasma silymarin concentration–time profiles

X. Drug administration and sample extraction procedure for tissue distribution studies and pharmacokinetic study

Wistar rats weighing (210±10) g were fasted for overnight before the experiments but allowed free access to water. The rats in each group was administered a single dose of silymarin-entrapped xyloglucan microspheres and standard silymarin in phosphate buffer saline (pH 7.4). The procedures employed in this study were approved by Institutional Animal Ethics Committee (IAEC) of Smriti College of Pharmaceutical Education, Indore by using registration No: IAEC/SCOPE/08-09/20. Blood samples were collected from retro orbital at predetermine times intervals and centrifuged at 10,000 rpm for 10 minutes at room temperature. The plasma was separated and stored at −20 °C until analysis. The same method was followed in all cases at an interval of (control, 1, 2, 4, 6 and 24 hrs) during study. Following the blood collection, animals were killed and different organ like heart, liver, spleen, lung, brain, and kidneys were quickly removed. Tissue samples (0.5-1 g) were homogenized with phosphate buffer saline (pH 7.4) solution and separated by centrifugation at 10,000 rpm for 10 minutes at room temperature. The sample was mixed with acetonitrile and supernatant was separated and stored at −20 °C until analysis. The same method was followed in all cases at an interval of (control, 1, 2, 4, 6 and 24 hrs) during study.

The maximum plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}) were obtained directly from the plasma concentration-time

data. The area under the plasma concentration-time curve from time zero to infinity (*AUC*) was calculated according to the linear trapezoidal rule. Overall targeting efficiency (TE), targeting index (TI) and relative overall targeting efficiency (RTE) of silymarin microspheres were calculated (Eq...4, 5 and 6) and compared with those of pure silymarin solution to evaluate the target ability of silymarin microspheres.

XI. Tissue distribution studies

Tissue distribution study of optimized formulation was performed in wistar rats in order to find out its targeting potency of formulation to the liver.

The area under the concentration–time curve (AUC) was calculated using the linear trapezoidal rule and extrapolated to infinity by dividing the last measurable concentration by the elimination rate constant. Overall targeting efficiency (TE^C), targeting index (TI^C) and relative overall targeting efficiency (RTE^C) (Jian and coworker (2002). [109] of silymarin microspheres were calculated and compared with those of pure silymarin solution to evaluate the target ability property of silymarin microspheres. Overall targeting efficiency (TE^C), targeting index (TI) and relative overall targeting efficiency can be calculated by following formula.

• OVERALL TARGETING EFFICIENCY (TE^c)

$$TE = \frac{(AUC0\rightarrow x)\,i \times 100\%}{\sum_{i=1}^{n} (AUC0\rightarrow x)\,i} \qquad \text{Eq.....4}$$

• TARGETING INDEX (TI)

$$TI \frac{(AUC0\rightarrow x)\ \text{SILY-MICROSPHERE}}{(AUC0\rightarrow x)\ \text{SILY-SOLUTION}} \qquad \text{Eq.....5}$$

• RELATIVE OVERALL TARGETING EFFICIENCY

$$RTE = \frac{TE\ \text{SILY-MICROSPHERE} - TE\ \text{SILY-SOLUTION}}{TE\ \text{SILY-SOLUTION}} \times 100\% \qquad \text{Eq.....6}$$

3. Results and discussion

3.1 Characterization of microsphere

I. Drug polymer interaction

The differential scanning calorimeter analysis of xyloglucan and the physical mixtures shows that there was no significant interaction b etween drug and polymers as shown in figure 3

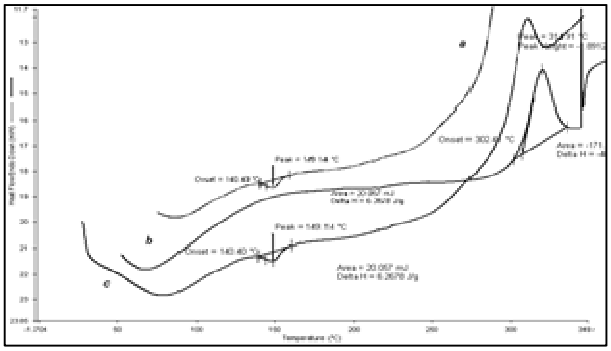

Fig 3: DSC of xyloglucan and silymarin blend

a = Pure silymarin
b = Xyloglucan
c = blend of pure silymarin and xyloglucan

II. Particle size-

The particle size of the microspheres was determined by using a Zetasizer (Malvern ZC, 90, UK). The average particle size of microspheres increased with increasing the polymer concentration. The mean diameter of microspheres was found to be 2.0±93, 7.8±21, 1.1±23 µm for 2, 3 and 4% of polymer concentration, respectively.

III. Effect of polymer drug ratio:

The entrapment efficiency was increased progressively with increase in xyloglucan concentration, which resulted in the formation of larger microspheres entrapping greater amounts of the drug. This may be attributed to the greater degree of cross-linking as the quantity of Xyloglucan increased on increasing the concentration of polymer the entrapment efficiency of formulations was 68.05±0.9, 75.85±1.4 and 74.05±2.1% for formulation S1, S2, and S3, respectively. The results are given in Table 2

On increasing the polymer concentration, the degree of swelling of microspheres was found to be increased and degree of swelling was 1.07±0.20, 1.29±0.18 and 1.56±1.15 for formulation S1, S2, and S3, respectively. The increasing swelling was

attributed to the interaction between free accesses of water to the xyloglucan hydroxyl group, which increased swelliablity of microspheres. The results are given in Table 2

IV. Effect of glutaraldehyde concentration:

The effect of cross linking agent on particle size was also determined. With increasing the cross linking by glutaraldehyde *i.e.*, 1 to 2 ml, particle size was found to be increased 1.0±10, 4.0±41, and 1.7±15 µm for formulation S4, S5 and S6 respectively. This is attributed to the fact that with an increase in the amount of glutaraldehyde, shrinkage of particles might have occurred

Table 2: Effect of drug polymer amount

S. no.	Drug: polymer	Average particle size (µm)	% entrapment	Swelling index	% yield	% cumulative drug release in PBS (pH 7.4) (up to 8hrs)
S1	1:2	2.0±93	68.05±0.9	1.07±0.20	73.13±1.2	78.06±0.23
S2	1.3	7.8±21	75.85±1.4	1.29±0.16	74.52±2.2	74.89±0.10
S3	1.4	1.1±23	74.05±2.1	1.56±1.15	78.05±1.0	73.98±0.11

Table 3: Effect of glutaraldehyde concentration

S no	Amount of glutaraldehyde (ml)	Average particle size (µm)	% entrapment	Swelling index	% yield	% cumulative drug release in PBS (pH 7.4) (up to 8hrs)
S4	1	1.0±10	71.32±2.6	1.51±0.40	74.42±1.3	74.59±082
S5	1.5	4.0±41	73.35±1.5	1.20±0.06	73.13±2.6	69.55±0.11
S6	2	1.7±15	68.05±1.2	0.95±0.74	73.93±1.6	68.65±0.54

Table 4: Effect of Stirring time

S. no.	Stirring time (min)	Average particle size (µm)	% entrapment	Swelling index	% yield	% cumulative drug release in PBS (pH 7.4) (up to 8hrs)
S7	15	2.8±89	70.25±2.2	0.87±0.90	75.05±1.1	74.58±0.85
S8	30	1.4±28	72.95±1.8	1.31±0.54	72.03±2.0	72.96±0.05
S9	45	5.4±82	73.01±2.3	1.42±030	69.87±1.5	73.95±1.44

leading to the formation of smaller particles. The results are given in Table 3

Up to 1.5 ml of optimal concentration of cross-linking agent the entrapment efficiency of formulation was found to be increased but after 1.5 ml glutaraldehyde concentration, the entrapment efficiency was decreased, which could be due to the increased cross linking of microspheres, which form more rigid particle and the free volume space within the matrix may decreases, resulting in reduced entrapment efficiency. The entrapment efficiency for formulation S4, S5, and S6 was found to be 71.32±2.8, 73.35±1.5 and 68.05±1.2%, respectively. The results are given in Table 3

Because of cross-linking with glutaraldehyde, the overall swelling of polymer was found to be decreased significantly. The cross-linking interferes with free access of water to the xyloglucan hydroxyl group, which in turn reduces the swelling properties of the cross-linked polymer; hence, the cross-linking of the xyloglucan formulation depended on the glutaraldehyde concentration. On increasing the glutaraldehyde concentration the swellability was found to be decreased 1.51±0.40, 1.20±0.08, 0.95±0.74 for formulation S4, S5, and S6, respectively. Table 3

V. Effect of span 80 concentration

Span 80 was used to facilitate the stable dispersion of the polymer in oil. With a fixed rotational speed, the stability of the dispersion of a particular polymer system depends on the concentration of emulsifier. An optimal concentration of emulsifier is required to produce

the finest stable dispersion, below this concentration the dispersed globules/droplets tend to fuse and produce larger globules because of insufficient lowering in interfacial tension.

VI. Effect of Stirring Time

The stirring speed and stirring time was observed to produce great influence on the size as well as drug entrapment efficiency of microspheres because as on increasing the speed as well as time of stirring the shear stress to the dispersion of xyloglucan in organic solvent increases which increases the drug entrapment efficiency due to increase surface area on the reduction of the size of microspheres.

The particle size was found to be 2.8±89, 1.4±28, and 5.4±82 µm for formulation S7, S8 and S9, respectively. On increasing the stirring time, the entrapment efficiency (%) was ranging between 70.25±2.2, 72.95±1.8, and 73.01±2.3% for formulation S7, S8 and S9, respectively. The results are given in Table 4

VII. *In vitro* release studies

The *in vitro* drug release studies were carried out for all formulation using phosphate buffer saline (pH 7.4) as the releasing media. *In vitro* drug release study was performed in phosphate buffer saline PBS (pH 7.4) at 37±0.2 ^{0}C. On increasing the concentration of polymer the release rate of drug from microspheres was decreased this could be because of the thickness of polymer layer was increased so the diffusion distance of drug to diffuse out from the microspheres was decreased. The cumulative drug released (%) observed in PBS (pH 7.4) was 78.06±0.23, 74.89±0.10 and 73.98±0.11% for formulation S1, S2, and S3, respectively. The results are given in Table 2

Glutaraldehyde causes cross linking by reacting with hydroxyl group of galactose, glucose and xylose unit of xyloglucan, thus interfering with free access of water to the hydroxyl group of xyloglucan. This significantly reduces the release rate of microspheres. In PBS (pH 7.4) the % cumulative drug release was 74.59±082, 69.55±0.11 and 68.65±0.54% for formulation S5, S6, and S7, respectively. The results are given in Table 3

The drug release was increase from 74.58±0.85, 72.96±0.05 and 73.95±1.44 in up to 8 when the stirring time was increased from 15 to 45 min which could be decreasing the size of microspheres. The result are given in Table 4

The result obtained *in vitro* release studies were plotted in different model of data treatment as follows

- ✓ Cumulative percent drug released V/s time (Zero order rate kinetics)
- ✓ Log cumulative percent drug retained V/s time (First order kinetics)
- ✓ Cumulative percent released V/s square root of time (Higuchis classical diffusion equation).

The kinetic values obtained for different formulation indicated in Table 5 for formulation S2, S5 and S8, respectively. The regression coefficient for the formulation S2, S5 and S8 for zero order plots were found to be 0.9958, 0.996 and 0.9971, respectively. The regression coefficient values for the formulation S2, S5 and S8 for first order plots were found to be 0.9598, 0.9878 and 0.9881, respectively

S2, S5 and S8 for first order plots were found to be 0.9598, 0.9878 and 0.9881, respectively.

These results indicate that formulation followed zero order kinetics.

The plot of cumulative percent drug released V/s square root of time was observed that formulation S2, S5 and S8 with regression coefficient of 0.9722, 0.9878 and 0.9902 the formulation S8 followed higuchi matrix suggesting diffusion controlled release.

Table 5: *In vitro* release studies of different Formulation

S. no	Time (hr)	Root T	Log T	S2 formulation		S5 formulation		S8 formulation	
				Cum. (%) drug release	Cum. (%) drug retained	Cum. (%) drug release	Cum. (%) drug retained	Cum. (%) drug release	Cum. (%) drug retained
1	1	1	0	23.67±0.02	76.33	18.89±0.12	81.11	22.37±0.06	77.63
2	2	1.414	0.301	31.67v0.04	68.14	28.96±0.21	71.39	31.81±0.9	68.19
3	3	1.732	0.447	39.90±0.06	60.10	35.93±0.14	62.07	38.68±0.4	61.32
4	4	2	0.602	43.79±0.08	56.21	41.65±0.31	55.35	46.31±0.4	53.69
5	5	2.236	0.698	51.16±0.09	48.84	49.48±0.21	48.52	52.66±0.6	47.34
6	6	2.449	0.778	58.80±0.05	41.20	54.29±0.32	41.71	60.65±0.7	39.29
7	7	2.645	0.845	66.71±0.11	33.28	61.43±0.12	36.57	67.71±0.12	32.29
8	8	2.828	0.903	74.89±0.10	25.11	69.55±0.11	28.45	72.96±0.05	27.04

Table 6: Regression coefficient of S2, S5 and S8 Formulation in Different plot

S. no	Formulation Code	Zero Order	First order	Higuchi
		r^2	r^2	r^2
1	S2	0.995	0.959	0.972
2	S5	0.996	0.987	0.987
3	S8	0.997	0.988	0.990

Table 7: Overall targeting efficiency (TEc) of silymarin entrapped in microsphere and standard silymarin

Tissue	Heart	Liver	Spleen	Lung	Kidney	Brain
SILY-SOLUTION	9.6	30.71	12	9.8	17.33	10.6
SILY-MICROSPHERE	10.7	74	6.7	8.4	16.4	8.7

Table 8: Comparison of targeting parameters between silymarin entrapped in microsphere and standard silymarin

Tissue	Heart	Liver	Spleen	Lung	Kidney	Brain
Ti^c	2.4	1.87	0.78	1.19	0.78	1.6
RTE^c (%)	9.3	77.83	-44.16	14.28	-9.17	-17.92

Table 9: AUC data of silymarin entrapped in microspheres (Tissue distribution)

S. No	Organ	Silymarin entrapped microspheres AUC (µg/hr/g)	Standard silymarin
1	Heart	64.27±0.02	82.18±0.02
2	Lung	77.74±0.06	46.64±0.04
3	Kidney	109±0.04	44.27±0.03
4	Spleen	44.67±0.04	77.17±0.04
5	Liver	358.1±0.01	193.6±0.02
6	Brain	31.32±0.04	50.27±0.07

VIII. *In vivo* study

Tissue distribution study of optimized formulation was performed in wistar rats in order to find out its targeting potency of formulation to liver. Tissue distribution profiles of silymarin in different tissues including heart, spleen, lung, liver, brain and kidney were measured after oral administration of silymarin microspheres and standard silymarin to rats. The result revealed that the higher amounts of silymarin-entrapped microspheres were found to accumulate in liver as compared to free silymarin. The results are given in Figure 4

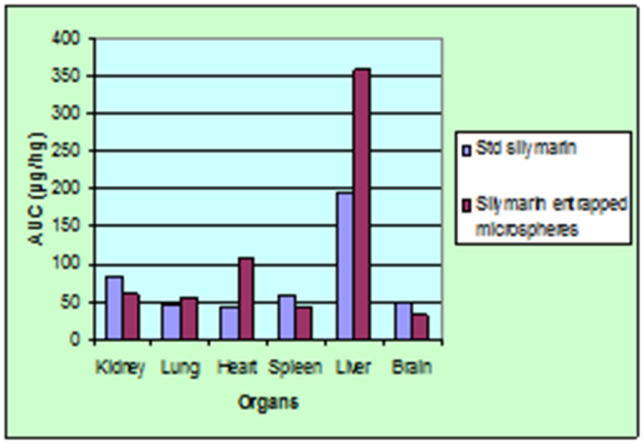

Fig 4: AUC graphs of silymarin entrapped microspheres and standard silymarin

The relative overall targeting of xyloglucan microspheres and standard silymarin solution was found to be 9.3, 75.83, -44.18, 14.28, -9.17 and -17.92 for heart, liver, spleen, lung, kidney and brain, respectively. The maximum relative targeting of silymarin-entrapped xyloglucan microspheres in the liver was 75.83 %.
(Table 8). On comparison of the overall targeting efficiency (TE^c) of silymarin entrapped xyloglucan microspheres and standard silymarin solution, the liver targeting efficiency of microspheres greatly improved. The liver AUC of silymarin microspheres was 1.85 fold greater than standard silymarin. The overall targeting (TE^c) of formulation was enhanced from 30.71 to 54%. (Table 7 and 9).
The AUC of silymarin entrapped xyloglucan microspheres were found to be higher in the liver, which could be due to xyloglucan which have galactose residues in its side chain recognized by asialoglycoprotein receptor (ASGPR) on hepatocyte, the specific interaction of xyloglucan with hepatocyte would further enhance the maintenance of the function. Xyloglucan is hydrophilic polymer, and it prevents opsonization of Drug delivery system in the blood stream and subsequent clearance by macrophages.
The overall result indicates that's the xyloglucan microspheres have ability to target the drug to the liver. The system would also provide sustained value of silymarin for longer period as time in the liver. The hydrophilic nature of system would be prevent opsonization and enhance the retention of formulation in systemic circulation and could improve the therapeutic index and patient compliance.

IX. Pharmacokinetics parameter

The *in vivo* pharmacokinetic parameters of silymarin entrapped xyloglucan microspheres after oral administration in rats, it determined by analytical HPLC method. The result shown in Table 10
Figure 5 show that the maximum plasma concentration of silymarin entrapped xyloglucan microspheres peaked at about 6 hr after dosing, and the $AUC_{0\text{-}t}$ was 131.70. The $AUC_{0\text{-}t}$ of standard silymarin was found to be 53.87 for 4 hr. These results indicated that the oral administration of silymarin-entrapped microspheres would maintain longer effective drug concentration in plasma than the concentrations induced by standard silymarin.

Table 10: Pharmacokinetic parameter of silymarin microsphere or standard silymarin

Pharmacokinetic parameter	Std silymarin	Silymarin microspheres
C_{max} (µg/ml)	19.21	39.45
t_{max} (hr)	4	6
Area under curve (µg/hr/g)	53.87	131.70

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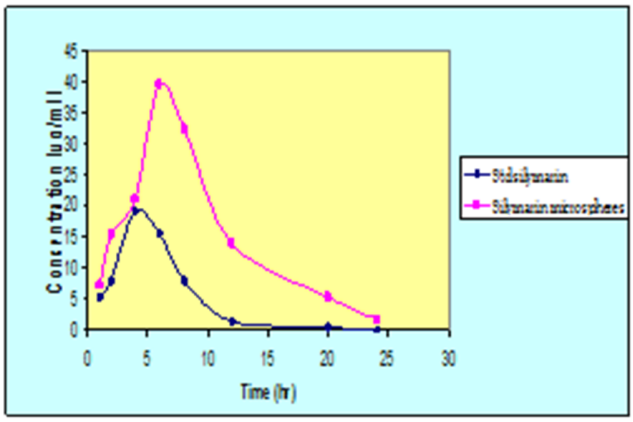

Fig 5: Concentration time profiles of standard silymarin and silymarin entrapped xyloglucan microspheres

4. Conclusions

Xyloglucan microspheres of silymarin were prepared by emulsification method using glutaraldehyde as cross-linking agent. With increase in concentration of xyloglucan there, size of microspheres as well as drug entrapment of drug was increased. With increase in amount of cross-linking agent (glutaraldehyde), the drug entrapment of formulation was found to be decreased. In addition, with increase in concentration of glutaraldehyde, the release rate of drug from microspheres is significantly reduces. The *in vivo* studies on rats were performed for tissue distribution and plasma drug concentration. The maximum concentration of drug was found in hepatic tissue at various time points suggesting the accumulation of drug through xyloglucan microspheres in liver. The result suggests that microspheres of silymarin can be successfully prepared by the isolated TSP through emulsification method. The prepared microsphere is able to achieve liver targeting that is evident from the result. Thus, the polymer could serve as a new effective drug delivery system for the liver targeting.

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