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Tamarind Seed Polyose : A Potential Polysaccharide for Sustained Release of Verapamil Hydrochloride as a Model Drug

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Tamarind seed polyose (TSP), a polysaccharide obtained from the seeds of Tamarindus indica was studied for sustaining the release of verapamil hydrochloride. The release pattern was compared with matrices of other polysaccharide polymers such as ethyl cellulose, hydroxyethyl cellulose and hydroxypropylmethyl cellulose, as well as the commercially available sustained release tablets (Isoptin SR). Formulation was also done by substituting part of the polymer with ethylhydroxyethyl celluose for enhancement of the drug release. The *in vitro* release of the drug from the matrices prepared were studied using the Sartorius Dissolution Simulator. Two formulations, found comparable to Isoptin SR were subjected to *in vivo* studies in rabbits. The results indicated a good correlation between *in vitro* and *in vivo* studies.

POLYSACCHARIDE polymers and their derivatives have been the polymers of choice as the rate controlling carriers for sustained release drug delivery[1]. A large number of natural and semisynthetic polysaccharides have been successfully used. Tamarind seed polyose[2,3] is an oligosaccharide which is obtained from the seeds of Tamarindus indica (Leguminosae). A haemostatic dressing and fallopian tube occluding agent[5] have been developed in C.D.R.I. using this polysaccharide. It has also been used as a binder in tablets, emulsifying agent[6] and for suspending insoluble powders[7]. The study which follows is an investigation of this polysaccharide as a polymer for sustaining the release of verapamil hydrochloride[8,9], an antihypertensive, antiarrhythmic, as a model drug. The comparison was made with other commercially available polymers and a market sustained release preparation of verapamil hydrochloride. Some of the tablets found satisfactory in *in vitro* studies were subjected to *in vivo* studies in rabbits by 3x4 radomised block design and pharmacokinetic parameters such as Cmax, tmax,AUC(0-8 hours) were calculated. The marketed sustained release preparation was choosen as reference formulation.

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MATERIALS AND METHODS

Verapamil hydrochloride was kindly provided by Dabur (India) Pvt. Ltd., Ghaziabad, India. The polymers used were hydroxypropylmethyl cellulose (HPMC), Cat. No. 29,441-1, Aldrich Chemicals Co., USA; hydroxyethylcellulose (HEC), Fluka Ag, Switzerland; ethyl cellulose (EC), and Ehylhydroxyethyl cellulose (EHEC) were obtained from BDH, England. Tamarind seed polyose (TSP) was prepared on laboratory scale[2] at the CDRI. All reagents used were of analytical grade. The chromatographic solvents were of HPLC grade (E. Merck India Ltd.). The verapamil hydrochloride sustained release tablet (Isoptin) was purchased from the market [MSRT].

Preparation of Matrices

The series of matrices with different drug : polymer ratios containing 180 mg of verapamil hydro-

chloride were prepared on a Korsch single punch tabletting machine using magnesium stearate (1% w/w) as lubricant and keeping angle of repose 40±5°. The hardness, surface area and % friability of the tablets were determined by Monsanto hardness tester, screw gauge, and friability test apparatus respectively. Percent Drug contents were calculated by comparing O.D. [at 278 nm against methanol; as blank on a Shimadzu UV/Vis 260 Spectrophotometer] of the methanolic extract of powder equivalent to 5 mg of verapamil hydrochloride (from powdered 5 tablets) after proper dilution with methanol with that of the reference standard.

In vitro release studies

Dissolution studies of the formulated sustained release tablets were carried out using the Sartorius Dissolution Simulator (Type SM 16751 Fab. Nr. 2339, Sartorius-GMBH, Gottingen, Germany) which simulates the pH, volume of liquid, time of stay, temperature and the mechanical agitation or mixing in the GI tract.

The study for each formulation and commercially available sustained release tablets of the drug was carried out in two phases,(i) at pH 1.2 for 4 followed by replacement of the buffer by (ii) pH 7.4 buffer for 7. This was done to extend the duration of study to 11 hours time period. Four ml samples were withdrawn at regular time intervals and automatically replaced by equal volume of fresh buffer. Drug contents were assayed by UV at 278 nm. The experiment were repeated 3 times and average values were taken. Standard deviation was less than 4% for all the formulations studied.

In vivo study design

Rabbits weighing 2-2.8 kg and at least 8 weeks of age, were obtained from C.D.R.I. animal house. Drinking water and a commercial laboratory diet was made available throughout the study. All animals were fasted for 12h before drug administration were administered with a dose equivalent to 60 mg orally by a stomach tube. About 1 ml blood samples were drawn from the marginal ear vein of each rabbits before administration and 0.5, 1, 1.5, 2.0, 2.5, 3, 4, 5, 6, 7, 8 and 24 h. after. The blood was taken into herparinized tubes which were centrifuged to separate the plasma fraction and the plasma was immediately processed to extract out the drug. Verapamil was analysed using a HPLC method.

The HPLC instrument consisted of Perkin Elmer 250 solvent-delivery system, a Rheodyne (Cotati, CA, USA) model 7125 injector with a 20 µl loop and a Perkin Elmer model 235 diode array detector. Separation was carried out on a ODS (E. Merck 5 µm particle size) column (250 mm x 4 mm ID). The column effluents were monitored at 280 nm. Chromatograms were recorded on a G. P. 100 printer plotter. Acetonitrile : phosphate buffer (0.1M,pH 3) 40:60 was deaerated and used as mobile phase with a flow rate of 1 ml/min. 250 µl of the serum was mixed with 100 µl of 10 n NaOH followed by 3 ml heptane, vortexed for 3 and centrifuged for 5 min at 3000 rpm. The organic layer was separated and extracted with 3 ml 1N HCL. The aqueous layer was separated, treated with 1 ml 10 n NaOH and extracted with heptane. The heptane layer was dried and reconstituted in 50µl of mobile phase and 20 µl were injected. Peak heights were measured and plasma concentrations in the unknown samples were established using calibration curves. The calibration curves were prepared in rabbit plasma with triplicate standard samples of verapamil hydrochloride. The detection limit of the above procedure was 100 ng/ml with linearity range of 100 ng - 1550 ng. The accuracy and reproducibility of the method were 5.0% and 8.7% respectively. The recoveries were found to be more than 90%.

Data analysis

The significant difference among the formulations were determined by one way ANOVA. Intra subjects variations were calculated by 't' test. The level of significance has been fixed at $P < \text{or} = 0.05$.

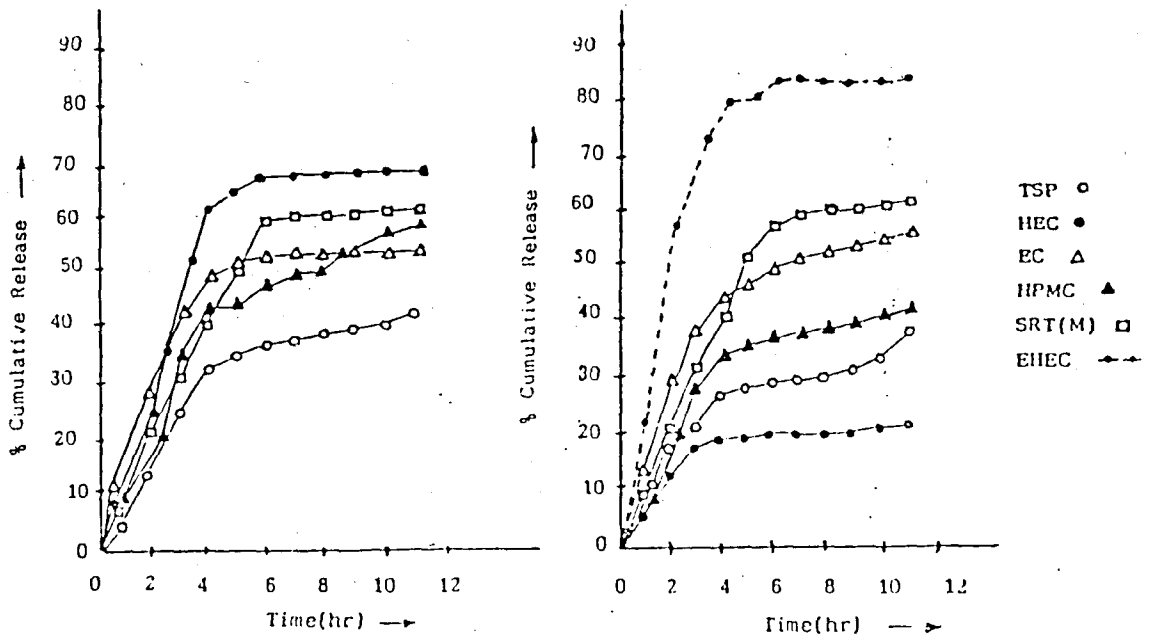


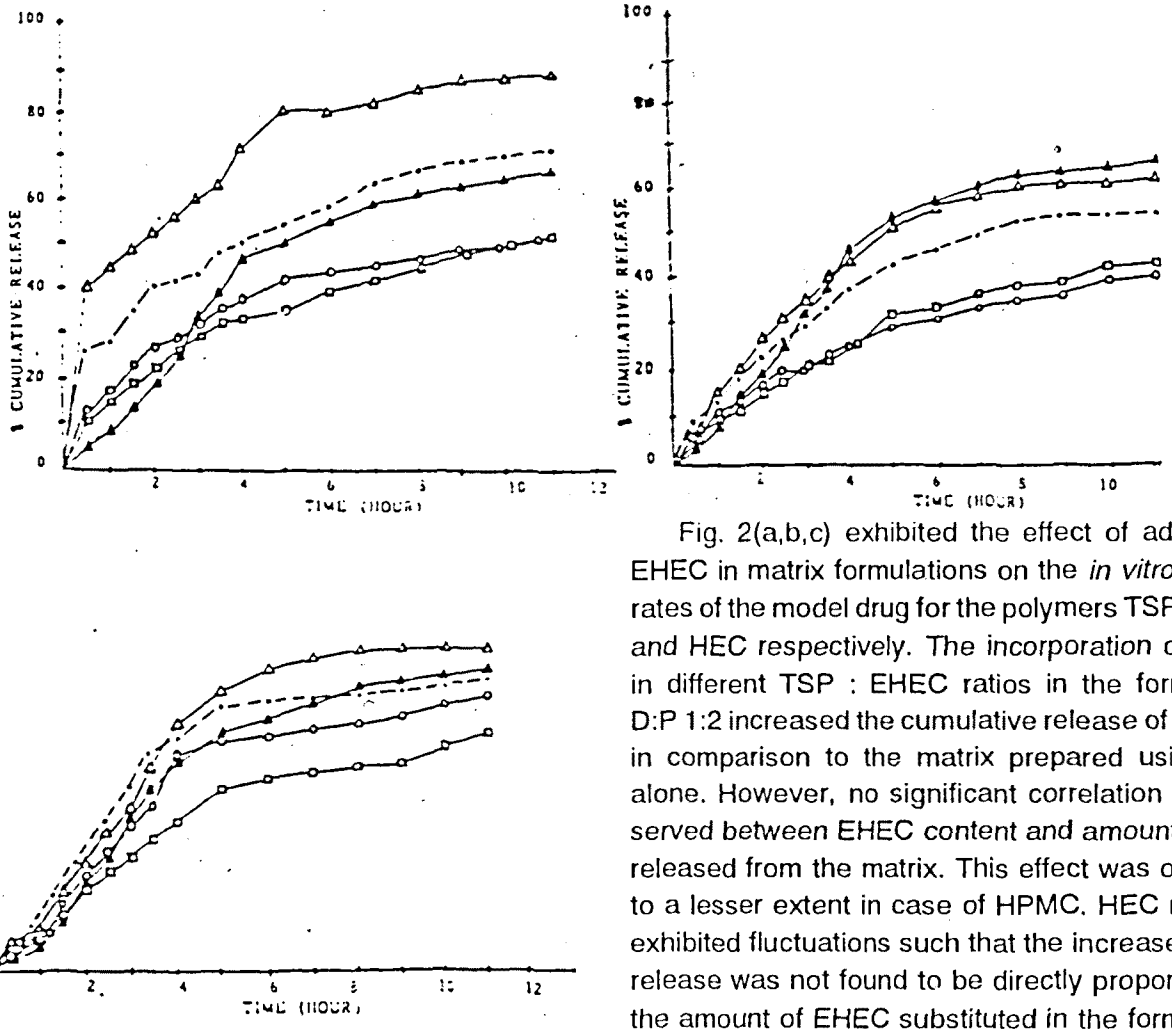
Fig. 1A & 1B : In vitro release profiles of verapamil hydrochloride matrices made from 1:1 and 1:2 ratios of drug : polymer respectively

RESULTS AND DISCUSSION

The first series of formulations of verapamil prepared by the method of direct compression consisted of two ratios of drug to polymer namely 1:1 and 1:2. These were subjected to the different tests on physical parameters like weight variation, hardness, surface area and percent friability. It was found that weight of matrices of drug to polymer (D:P) ratio 1:2 was in the range 0.5320 to 0.5402 g and those for the D:P ratio 1:1 in the range 0.3550 to 0.3610 g. The average weight of MSRT was found to be 0.78 g. The hardness and percent friability of the formulated matrices irrespective of the D:P ratio averaged about 4:1 kg/cm^2 and 0.099 % respectively. The same parameters of the MSRT were 12.0 kg/cm^2 and 0.004% respectively, the mean value of hardness being very high and the reverse being the case for friability. The mean surface area of the matrices of D:P ratio 1:1 was 255.77 mm^2, for D:P ratio 1:2 was 477.10 mm^2 and for the MSRT 433.03 mm^2. The drug content was 99-101% in all the tablets tested, i.e., formulated as well as marketed preparations.

Invitro release profiles of formulated and marketed preparations in terms of percent cumulative release versus time are shown in Fig. 1.A (for the D:P ratio 1:1) and Fig. 1B (for the D:P ratio 1:2). For the D:P ratio 1:1 (50% drug loading), as compared to the MSRT, the polymer HEC sustained the release of model drug to a lesser extent whereas release was lesser in the case of HPMC EC and TSP. An increase in the polymer content in the matrices (from D:P 1:1 to D:P 1:2) resulted in a decrease in the amount of drug released from matrices over the period of study. In case of HEC, an increase in polymer content from D:P 1:1 to 1:2 resulted in a drastic decrease in the cumulative release of drug. This effect was less pronounced in case of EC, followed by HPMC and TSP. A matrix formulation with EHEC in the D:P ratio 1:1.5 released the entire amount of drug incorporated within a period of 4-5 h of *in vitro* study. Furthermore, as evident from the figure, in all formulated tablets and to a lesser extent in case of MSRT, there was a leveling off of the curve when the pH changed from the acidic side (pH 1.2) to the alkaline side (pH 7.4). The decrease

Fig 2A, 2B & 2c : In-vitro release profiles of verapamil hydrochloride matrices made from drug polymer ratio 1:2 using different ratios of TSP/HPMC/HEC and EHEC as polymer formultion : A (•), B (˚), C (Δ), D (□) and MSRT (Δ)



in the release rate of the drug must be due to the high pH dependency of solution of the model drug[10].

On the basis of the above observations for matrices in the D:P ratio 1:2 formulated using the polymers HEC, HPMC and TSP, a part of the polymer was substituted by EHEC. Four combinations of each polymer with EHEC namely A, B, C and D were formulated. The details of the four combinations have not been provided nor the physical parameters of the matrices, as they have been patented[11]. The drug content in all the formulated matrices was 99-101%. These matrices, with 33% drug loading, were subjected to an *in vitro* release study on the same pattern as for the above formulations.

Fig. 2(a,b,c) exhibited the effect of addition of EHEC in matrix formulations on the *in vitro* release rates of the model drug for the polymers TSP, HPMC and HEC respectively. The incorporation of EHEC in different TSP : EHEC ratios in the formulation D:P 1:2 increased the cumulative release of the drug in comparison to the matrix prepared using TSP alone. However, no significant correlation was observed between EHEC content and amount of drug released from the matrix. This effect was observed to a lesser extent in case of HPMC. HEC matrices exhibited fluctuations such that the increase in drug release was not found to be directly proportional to the amount of EHEC substituted in the formulation. Some combinations of TSP with EHEC, namely formulation C exhibited a good and continuous release profile over the duration of study. The decrease in the rate of drug release over the alkaline pH range was eliminated.

The linearity of the straight line determined from the coefficient of correlation R, was estimated for the *in vitro* data for all the matrices formulated as well as marketed preparations. The data was subjected to Higuchi equation[12], zero order[13] and first order[14] kinetics equations and the R value determined. The Tablet I shows the R values for different

Table -I

Co-efficient of Correlation for Zero and First Order Kinetic Models and the Higuchi Equation

		Zero order	First order	Higuchi Eq.
DRUG:TSP	1:1	0.9032	0.7225	0.9553
	1:2	0.9417	0.8677	0.9777
DRUG:HEC	1:1	0.8421	0.8376	0.9582
	1:2	0.8106	0.6435	0.8840
DRUG:EC	1:1	0.7991	0.6877	0.8884
	1:2	0.8829	0.7321	0.9497
DRUG:HPMC	1:1	0.9368	0.8042	0.9790
	1:2	0.8004	0.6412	0.9163
FORMULATIONS CONTAINING				
HEC/EHEC	A	0.9051	0.8078	0.9750
	B	0.8926	0.7232	0.9471
	C	0.9454	0.7885	0.9798
	D	0.9423	0.8320	0.9822
TSP/EHEC	A	0.9339	0.8721	0.9798
	B	0.9828	0.9658	0.9923
	C	0.9457	0.8802	0.9853
	D	0.9507	0.9258	0.9834
HPMC/EHEC	A	0.9638	0.8548	0.9941
	B	0.9476	0.8570	0.9865
	C	0.9688	0.8864	0.9954
	D	0.9323	0.8272	0.9785
SRT (M)		0.9391	0.7964	0.9843

order of release for the formulated and marketed preparations. For the all matrices studied, the value of R was maximum for the Higuchi equation, indicating that the percent cumulative release was directly proportional to the square root of time.

On the basis of this preliminary study, the two formulations, one prepared using HPMC (D:P, 1:2) and the other formulation C of TSP in combination with EHEC, were selected for 3x4 randomised cross over design studies in rabbits with the reference formulation.

Table - II

Mean Pharmacokinetic Parameters of Verapamil after Oral Administration of Different Sustained Release Formulations (Mean ± standard deviation values)

Formulation	$AUC_{(0\,8)}$ (ug h/ml)	Cmax (ug/ml)	Tmax (h)
SRTM	23.93 ±2.15	6.58± 1.19	2.38 ±1.25
Drug : (TSP+EHEC) 1:2	25.71 ±2.50	6.51 ±2.32	3.00 ±1.47
Drug : HPMC 1:2	22.57 ±4.71	6.57 ±1.69	3.25 ±1.94
Intra Formulation Variations (P =)	0.042	0.2898	0.0003
Inter Formulation Variations (P =)	0.2001	0.9976	0.0776

Table II shows Cmax, Tmax and AUC (0-8 h) for the 4 rabbits treated with the two formulations, and the marketed sustained release tablet of verapamil hydrochloride. These pharmacokinetic parameters were subjected to one way ANOVA and intra subject variations were calculated by 't' test. There was no significant difference in the mean AUC (0-8 h), Cmax and Tmax of three formulations administered in the same rabbit. It was found that AUC varied significantly among the rabbits while in Cmax values there was no significant difference between the rabbits. Tmax exhibited significant difference between the rabbits receiving same formulations. This was due to the high Tmax values obtained in the case of rabbit no. 2 in all the cases. Animals observed on dosing and on withdrawal of blood sample did not show distress or side effects arising from the procedure used on drug treatment.

In conclusion, these results indicate that TSP is a good polymer when used in an appropriate amount, as such or in combination with other commercially available polymers for sustaining the release of drug from matrices with high drug loading prepared even by the simple process of direct compression.

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