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Pharmacological screening of self-emulsifying drug delivery system containing cyclosporine for solubility enhancement

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Abstract
In the current research, Self-Emulsifying Drug Delivery system (SEDDS) for Cyclosporine is developed and evaluated for feasibility of oral administration. The objective behind the research was to increase the solubility of cyclosporine through Self Emulsifying Drug Delivery System that will increase the bioavailability and reduce the side effects of the drug. The SEDDS was prepared by hot homogenisation method and evaluated for droplet size, zeta potential, polydispersity index, drug loading, *in-vitro* study and *in-vivo* study. *In-vivo* studies for cyclosporine loaded self-emulsifying drug delivery system were carried out in albino rats and the pharmacokinetic parameters i.e. Cmax, $T_{1/2,}$ AUC, V_d were compared with a marketed formulation, which indicated better results of the prepared formulation than the marketed one. So it is concluded that self-emulsifying drug delivery system hold potential as a drug delivery system for drug with poor aqueous solubility.

Keywords: Cyclosporine, bioavailability, hot homogenisation method, *In-vivo* study

Introduction
It is well known that the oral route is the first choice clinically for drug administration due to better patient compliance. However, most new chemical entities (approximately 60% of drugs) coming directly from chemical synthesis are poorly water-soluble [1] and a large fraction of them are even poorly permeable across the bio membrane in the gastrointestinal tract (GIT). Drug substances with low solubility and poor permeability exhibit extremely low bioavailability after oral administration, which may result in limited therapeutic potential and thus insufficient curative effects [2]. According to the definition of Biopharmaceutics Classification System (BCS) [3], these drugs are categorized as BCS IV. Consequently, BCS IV drugs yield the most challenging absorption problems [4] such as low drug dissolution, efflux by transporters in the gut wall, and first-pass effect by metabolic enzymes [5].
This study utilized cyclosporine A (CyA), which is a cyclic polypeptide containing eleven amino acids and used as immunosuppressive, as a model drug. CyA is classified as a BCS IV drug due to its physicochemical properties, including high lipophilicity, polar surface area, and molecular weight [6-8]. The factors impeding the absorption of CyA include the narrow absorption window in the upper gut, P-glycoprotein efflux from enterocytes, and extensive presystemic metabolism in the wall and liver [9-10]. To date, how to improve CyA's *in vivo* performance has been a widespread concern, and various oral drug carriers have emerged, such as solid dispersion [11-12], nanosuspension [13], liposomes [14], lipid NPs [15], On the other hand, these preparations are alcohol free delivery systems that only require low concentrations of surfactant, thus their toxicity is expected to be diminished compared to the dosage forms on the market. In this paper we aim to evaluate the oral bioavailability of cyclosporine loaded self-emulsifying drug delivery system and Neoral® was used as reference formulation.

Materials and Methods
Materials
Cyclosporine A was collected as a gift sample from Sandoz Pharma, Switzerland. Commercially available Neoral® was purchased from the local market. Stearic acid and Tween 80 were purchased from S.D. Fine Chemicals, Mumbai, India. All other chemicals were of reagent grade and purchased from S.D. Fine Chemicals, Mumbai, India.

Preparation of Self emulsifying drug delivery system (SEDDS)

Hot homogenisation method

The Self emulsifying drug delivery system of cyclosporine was prepared successfully by this method using stearic acid as lipid carriers, tween 80 as a surfactant, PVA as a stabilizer. In this method, stearic acid was melted above its melting point i.e. 70 °C and the drug Cyclosporine A was dissolved in it. The solution of Tween 80 and PVA was prepared in purified water and heated to the same temperature as that of drug solution. The drug lipid mixture was added drop wise to the hot aqueous solution of surfactant with continuous stirring to make o/w pre-emulsion. The mixture was homogenised at a speed of 1500 rpm for 1 hour. The mixture was then cooled down to the room temperature to give Self emulsifying drug delivery system. The resultant mixture was filtered through membrane filter and Self emulsifying drug delivery system of cyclosporine was obtained.

Optimisation of Batch

The Self emulsifying drug delivery system of cyclosporine was prepared by hot homogenisation method using 3^2 factorial design. Total 27 batches (F-1 to F-27) were formulated by taking different concentrations of lipid (Stearic Acid), surfactant (Tween 80), and stabilizer (Polyvinyl Alcohol). All batches were evaluated for average particle size, entrapment efficiency, drug loading and percentage yield. Based on evaluation, Batch F-10 was selected as optimized batch on the basis of the smallest particle size, high entrapment efficiency and high drug loading.

***In-vivo* Studies**

Male albino rats (250-300 g) were utilized for *in-vivo* experimental studies. Optimized batch F-10 was selected for *in-vivo* studies on the basis of their average particle size, entrapment efficiency, drug loading and percentage yield. All the animal studies were conducted in accordance with the protocol approved by the Institutional Animal Ethical Committee of Moradabad Educational Trust Group of Institutions Faculty of Pharmacy, Moradabad (Registration No. METGI/FOP/CPCSEA/ 2018/05).

Experimental Design

The animals were divided into four groups, each group containing six animals. The rats were fasted overnight and then each group was given a different cyclosporine A formulation. The first group was treated as control and was fed with PBS solution (pH 7.4) by oral route. Second group was treated with a single dose of plain cyclosporine A suspension in buffered saline solution (pH 7.4). Third and fourth groups were treated with a single dose of cyclosporine loaded SEDDS (F-10) in buffered saline solution (pH 7.4) and Neoral (marketed formulation) by oral route. The normal human dose of Cyclosporine is 5 mg/kg of body weight. This dose was converted into animal dose by using the formula given below: - HED = Animal dose in mg/kg / (animal body weight in kg/human body weight in kg) $^{1/3}$, Where HED =Human equivalent dose.

Animal body weight = 0.280 kg
Human body weight = 60 kg

By using this formula, dose of Cyclosporine given to rats was obtained 280 µg. Hence, each of 3 formulations was administered at the dose of 280 µg Cyclosporine. Blood samples were withdrawn at 0.30, 1, 2, 4, 6, 8, 12, and 24 h after dosing. The blood samples were centrifuged, and 100 µl of plasma was separated and immediately frozen until required for analysis. The plasma samples were deproteinized with 100 µl of acetonitrile containing Para phenyl phenol, shaken on a vortex mixture, and centrifuged, and 20 µl of the supernatant was analysed by high-performance liquid chromatography method. Separation was carried out on reversed-phase column, and the column effluent was monitored using ultraviolet detector at 215 nm. The mobile phase was 45% acetonitrile in 0.1 M acetic acid (pH 3.5) at a flow rate of 1 ml/min.

Table 1: Peak plasma concentration in albino rats receiving different formulations

S. No.	Time (hr)	Plain drug	Optimised Batch (F-10)	Marketed formulation
1	0.30	3.0	3.5	4.5
2	1	3.5	4.0	4.9
3	2	2.8	3.2	3.0
4	4	2.7	2.8	2.5
5	8	2.4	2.6	2.4
6	12	1.8	2.3	2.0
7	20	1.5	2.4	1.8
8	24	1.2	1.7	1.6

Table 2: Blood cyclosporine concentration in albino rats receiving different formulations

Formulation	Time (hr)	Concentration (µg/ml)		Mean ± S.D
		1	2	
Plain drug	0.30	2.9	2.8	2.85 ± 0.70
	1	2.3	2.2	2.25 ± 0.070
	2	1.1	1.2	1.15 ± 0.070
	4	0.6	0.8	0.70 ± 0.141
	8	0.4	0.5	0.45 ± 0.70
	12	0.13	0.15	0.14 ± 0.141
	24	0.1	.09	0.09 ± 0.070
Optimised Batch (F-10)	.30	3.1	3.12	3.10 ± 0.021
	1	4.1	3.1	4.00 ± 0.141
	2	2.3	2.1	1.85 ± 0.070
	4	1.9	1.8	1.50 ± 0.141
	8	1.6	1.4	1.00 ± 0.141
	12	0.9	1.1	0.45± 0.141
	24	0.4	0.5	0.09 ± 0.070
Marketed formulation	.30	3.1	2.9	3.00 ± 0.141
	1	3.5	3.6	3.55 ± 0.070
	2	2.5	2.6	2.55 ± 0.070
	4	1.9	1.7	1.80 ± 0.141
	8	1.7	1.6	1.65 ± 0.70
	12	1.4	1.1	1.25 ± 0.212
	24	0.8	0.7	0.75 ± 0.070

Table 3: Mean pharmacokinetic parameters of cyclosporine following oral administration of three dosage forms

S. No.	Pharmacokinetic parameters	Plain drug	Optimised Batch (F-10)	Marketed formulation
1	AUC(µg*hours/mL)	8.67	33.64	44.01
2	C_{max} (µg/mL)	2.8	3.9	3.5
3	T_{max} (hrs)	4.08	2.5	1.92
4	T1/2	2.01	6.867	9.88
5	Kel	0.344	0.100	0.07
6	Vd	98.08	88.353	97.21
7	Total CL	34.58	8.91	6.89

Histopathological Evaluation

The organs including kidneys, liver, spleen, jejunum, ileum and colon were isolated immediately after sacrificing the animal. The tissues were washed with ice-cold saline and fixed in a 10% neutral buffered formalin solution. The tissues were then embedded in paraffin blocks and used for histopathological examination. Five-micrometer thick sections were cut, deparaffinised, hydrated and stained with hematoxylin and eosin. The histological sections were examined under microscope for the morphological changes. The morphometric analysis of the histological sections of kidneys were carried out for all the treatment groups. The diameters of the glomerular capillary tuft (CD) and the Bowman's capsule (BD) were measured with the help of an internal micrometer and the ratio CD/BD was calculated as an index of glomerular collapse.

Histopathological examination of the primary metabolizing organ (liver), spleen and the three intestinal segments viz. jejunum, ileum and colon were also carried out to study the presence of any inflammation after the administration of cyclosporine loaded Sedds. The control rats showed normal cytoarchitecture of hepatic parenchyma including Kupffer cells, hepatocytes and sinusoids (Fig. 1A). On the contrary, Sandimmune Neoral® treatment caused morphological alterations including disorganization of hepatic parenchyma with widespread cell swelling and congestion of sinusoids (Fig. 1B). However, no sign of morphological alterations was observed in the blank as well as cyclosporine loaded SEDDS treated rat livers (Fig. 1C and D).

The spleen histology of animals treated with Sandimmune Neoral® and blank as well as cyclosporine A loaded SEDDS was not different from normal, untreated ones in terms of the lymphoid tissue mass, the sinusoids and the appearance of RBCs. The inflammatory cells (lymphocytes) are inherently present in the normal intestinal segments of jejunum, ileum and colon since GIT is constantly challenged by the food and microorganisms. Inflammatory response in the intestine is normally evidenced by the appearance of inflammatory cells in the muscle layer encircling the mucosa. The mucosa showed no evidence of degeneration, suggesting that the SEDDS did not induce damage or initiate any inflammatory response in these tissues. A comparison of healthy tissue from untreated animals with that of animals treated with the Sandimmune Neoral® and blank as well as cyclosporine loaded SEDDS showed no sign of inflammation after 1 month treatment.

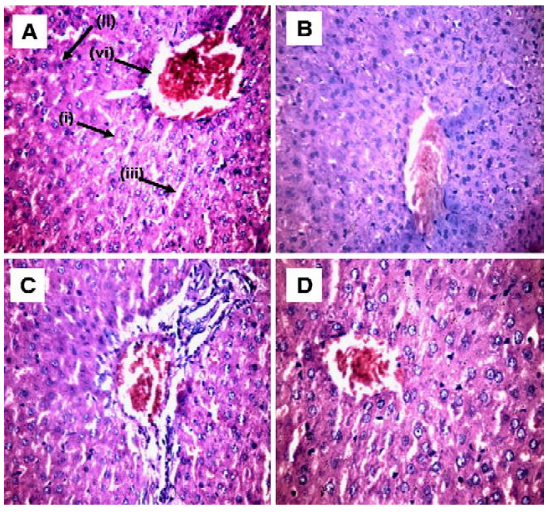

Fig 1: Histopathological evaluation

Results and Discussion

The current study showed the fact that cyclosporine-loaded self-emulsifying drug delivery system can be prepared with high entrapment efficiency using low stabilizer concentration. The pharmacokinetics of Cyclosporine incorporated into SEDDS following a single dose oral administration of 5 mg/ kg was investigated in albino rats using the marketed microemulsion (Neoral®) as reference. Cyclosporine A SEDDS formulations were administered freshly prepared and lyophilized in order to study the influence of particle size enhancement after the freeze drying. Regardless the formulation, fast absorption of CsA was observed within the first hour after drug administration, when it reached its peak levels, followed by a slower decline in concentrations, representing drug distribution and elimination processes. According to the pharmacokinetic data blood levels of the drug after oral Administration of this delivery system, part of the drug remains incorporated in the intact SEDDS is taken up from the intestinal lumen by the lymphatic transport and goes into the systemic circulation, where the drug reaches the target and delivery takes place. These particles were efficient in controlling the drug release in- vitro and *in-vivo*. All Pharmacokinetic parameters show favorable results. The self-emulsifying drug delivery system formulation showed significantly improved intestinal uptake and oral bioavailability as compared to Sandimmune Neoral®. Most importantly, the self-emulsifying drug delivery system formulation exhibited lower nephrotoxicity upon the chronic administration in rats as compared to the commercial formulation. So it is concluded that self-emulsifying drug delivery system hold potential as a drug delivery system for drug with poor aqueous solubility.

Conclusion

The *In-vivo* pharmacokinetic studies indicated potential of developed cyclosporine loaded Self emulsifying drug delivery system formulation for faster absorption and showed increased bioavailability of Cyclosporine The developed system has also shown potential of maintaining higher level of Cyclosporine for a longer period of time as compared to marketed one. From the present study, it can be concluded that Self emulsifying drug delivery system can be used efficiently for enhancing bioavailability of Cyclosporine via oral route.

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