Chapter 5
Pharmacokinetic Study

5.1. Pharmacokinetic analysis

The plots of drug plasma concentration Vs time were plotted for Cilostazol[1] and Tadalafil[2] after administration of their Oral Microemulsions, Drug-CDs inclusion complexes formulation orally and compared it with marketed formulations and pure drug. Wagner Nelson method was used for the calculation of the pharmacokinetic parameters[3-6] and the Pharmacokinetic parameters were calculated as follows:

5.1.1. PHRMACOKINCETIC STEPS FOR CALCULATING PHARMACO KINETIC PARAMETERS[3-6]:

1) **Maximum plasma concentration (C_{max}):** It was determined directly from the plasma concentration time profiles.

2) **Time to maximum plasma concentration (T_{max}):** It was determined directly from the plasma concentration time profiles.

3) **Area under the plasma concentration-time curve from time zero to t (AUC_{0-t}):** It was calculated by using trapezoidal rule. According to trapezoidal rule, the area under the curve from time t_2 to time t_1 is calculated by following equation:

$$AUC_{t_1}^{t_2} = \frac{C_1 + C_2}{2} \times (t_2 - t_1)$$

Where, C_1 and C_2 is concentration at time t_1 and t_2.

4) **Concentration at zero time (C_0):**

Plot time versus ln (conc.) graph on excel graph or plot time versus concentration in semi logarithmic graph paper. Extrapolate the terminal linear phase to zero. It gives intercept. The antilog of the intercept obtained by linear regression gives the concentration at zero time (C_0).

C_0 = Antilog (intercept).

5) **Elimination rate constant ($-K_{el}$):**

The plot of plasma concentration vs time was plotted on semi-logarithmic paper. The terminal portion (last three detectable concentrations) was essentially linear with the slope of $-K_{el}$. Calculate the slope of the terminal linear phase.

K_{el} = -slope × 2.303

6) **Elimination half life ($t_{1/2}$) :** It was determined by following equation:

$t_{1/2} = 0.693/K_{el}$

7) **Area under the plasma concentration-time from time zero to infinity ($AUC_{0-\alpha}$):** The trapezoidal rule written in its full form to calculate the AUC from t = 0 to t = α is as follows:

$$AUC_{t0}^{t\alpha} = \sum AUC_{tn-1}^{tn} + \frac{C_{pn}}{K_e}$$

Where,

C_{pn} = last observed plasma concentration at t_n and k = slope obtained from the terminal portion of the curve.

8) **Absorption rate constant (K_{ab}):** The terminal linear portion of the curve with slope-K_{el} was extrapolated to t=0. The actual plasma levels were subtracted from the corresponding concentrations on the extrapolated linear portions. This gave a series of residual concentration (Cr). The plot of natural log of residual concentration (ln Cr) vs time gave a straight line with slope –Kab.

9) **Absorption half life ($t_{1/2ab}$):** It was calculated as follows:

$(t_{1/2ab}) = 0.693/k_{ab}$

10) **Volume of distribution (V_d):** It is the volume in which drug would have to be distributed to produce the measured plasma concentration.

$$V_d = \frac{F \times G_0}{K_{el} \times AUC_{0-\alpha}}$$

(Fraction of administered dose G_0 absorbed following oral administration)

11) **Clearance (Cl):** It is the total volume of plasma from which the drug have been removed per unit time.

Clearance (Cl) = $(V_d \times 0.693)/t_{1/2el}$

12) **Cumulative drug eliminated at t time:** It is calculated as follow:

Drug eliminated = 0.434 K_{el} * t

13) **Fraction of drug absorbed at time t** $= \frac{C+K_{el} \times AUC_0^t}{K_{el} \times AUC_0^\alpha}$

14) **Total drug in plasma at t time** = original conc. in plasma calculated by HPLC method at t time + cumulative drug eliminated at t time.

15) **Residual concentration** = Ln conc – conc at t time in extraplotted linear eliminated line of graph.

16) **Area under momentum curve (AUMC):**

AUMC is the area under the curve of graph of C_p*t versus t.

17) **Mean residence time (MRT):**

MRT = CAUMC/CAUC

Where, CAUMC = Cumulative AUMC

CAUC = Cumulative AUC.

5.2. Pharmacokinetic Study

5.2.1. Pharmacokinetic study for Cilostazol Microemulsion (CME 2), Marketed formulation (Platoz-50) and Pure Cilostazol.

5.2.1.1. CALCULATION OF DOSE OF DRUG IN RABBITS

The dose of the drug in the rabbits was calculated, depending on the weight of the rabbits in mg/kg. By the following formula[7,8]:

HED (Human Equivalent Dose) for rabbit = 0.07 × Human dose of the drug

Where the rabbit weight was considered as 1.5 Kg.

The maximum dose of Cilostazol that can be given to human in single day is 100 mg. According to the above formula, the dose for the rabbits is calculated to be 4.66 mg/kg. In this study, the dose given to the rabbits is 7 mg/1.5 Kg of rabbit weight.

5.2.1.2. Pharmacokinetic study of Cilostazol Microemulsion

The pharmacokinetic study was performed in New Zealand rabbits weighing 1.0 to 1.5 kg. The animals were fasted over night prior to the experiment but had free access to water. The conventional tablet (Platoz-50) powder equivalent to 7 mg. of Cilostazol and pure Cilostazol (7 mg.) were taken and mixed with 1 ml of 0.5 % of sodium -carboxy methyl cellulose solution (Na-CMC). Oral microemulsion containing 7 mg/ml of Cilostazol was prepared and given orally to the rabbits as per the dose calculation method described in section 5.2.1.1. There were three groups containing eight rabbits in each group as two rabbits for placebo, two rabbits for pure Cilostazol, two rabbits for conventional tablet and two rabbits for oral microemulsion containing Cilostazol were selected. Blood samples were collected from the marginal ear vein at 1, 2, 4, 6, 12, 18 and 24 hrs after Cilostazol administration. The heparinised blood samples were immediately centrifuged at 40000 rpm for 15 minutes and separated plasma was stored at -20 °C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method described in section 3.1.3. and the drug plasma concentration values were determined from the calibration curve. The average drug plasma concentration of pure Cilostazol, conventional tablet and oral microemulsion are shown in Table 5.2.1.1. The average plasma drug concentration versus time profiles for all three formulation are given in Figure. 5.2.1.1 and the different pharmacokinetic parameters were calculated by using Wagner-Nelson method(Table 5.2.1.2.).

Table 5.2.1.1. :- Average plasma Cilostazol concentration after administration of pure Cilostazol, Conventional tablet and Oral microemulsion of cilostazol (CME 2) in rabbits.

Sr. No	Time (hour)	Plasma concentration in ng/ml ± SD		
		Pure Cilostazol	Conventional Tablet (Platoz-50)	Oral Microemulsion (CME 2)
1	0	00.00 ± 0.86	00.00 ± 0.27	0.00 ± 1.21
2	1	680.24 ± 3.42	945.15 ± 6.23	1226.8 ± 6.91
3	2	921.48 ± 5.86	1486.64 ± 9.06	2096.37 ±11.25
4	3	1150.6 ± 6.85	2126.48 ± 8.67	3050.51 ± 9.52
5	4	1021.11 ± 8.07	1735.91 ± 8.37	2586.26 ± 10.51
6	6	836.34 ± 7.44	1478.45 ± 7.25	1919.55 ± 6.43
7	12	601.44 ± 5.91	988.56 ± 5.14	1166.23 ± 5.11
8	18	450.56 ± 4.38	736.28 ± 4.29	908.63 ± 4.97
9	24	328.37 ± 1.49	529.82 ± 2.58	616.72 ± 3.33

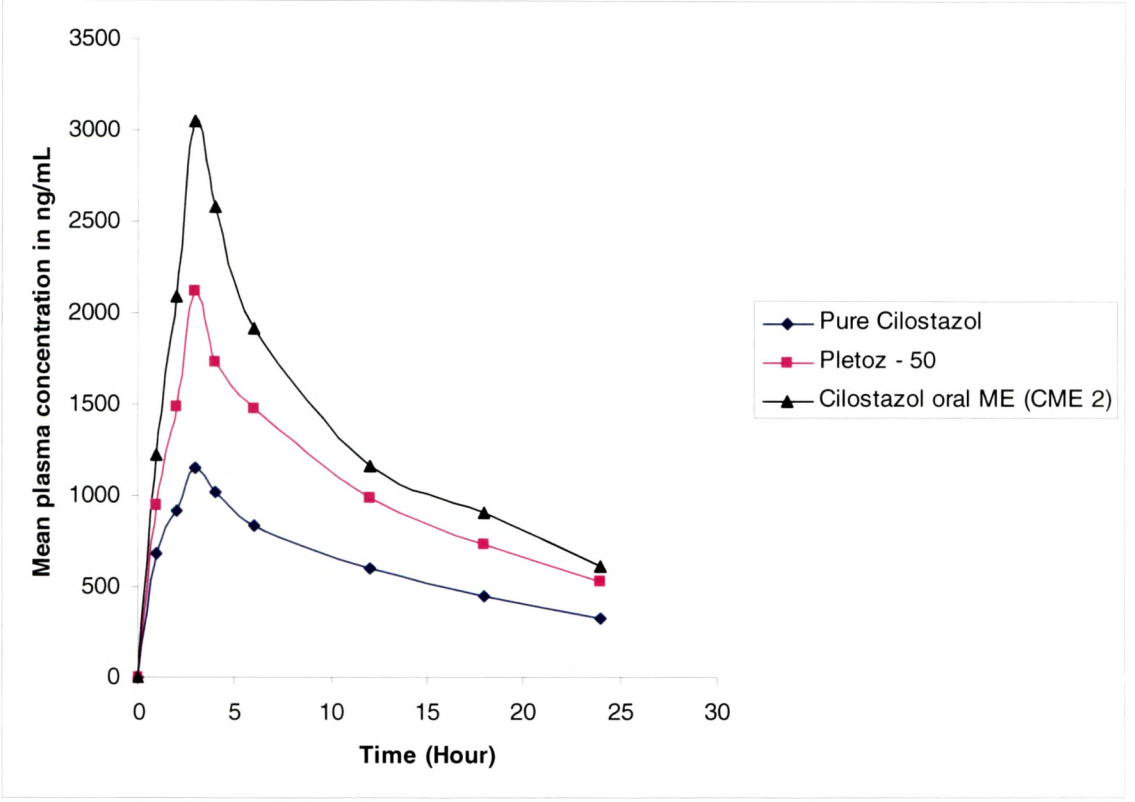

Figure 5.2.1.1. Comparison of pharmacokinetic profiles of Pure Cilostazol, Conventional tablet and Oral microemulsion of Cilostazol (CME 2).

Table 5.2.1.2. Different pharmacokinetic parameters calculated by Wagner-Nelson method.

Parameters #	Pure Cilostazol	Marketed formulation (Pletoz – 50)	Oral Microemulsion (CME 2)
C_{max} (ng/ml)	1150.96 ± 101*	2126.48 ± 185*	3050.51 ± 215*
t_{max} (h)	3.90 ± 1.32	4.00 ± 0.93	4.00 ± 2.16
k_a (h^{-1})	0.77 ± 0.006	0.63 ± 0.003	0.60 ± 0.008
k_{el} (h^{-1})	0.05 ± 0.002	0.06 ± 0.0032	0.07 ± 0.005
$T_{1/2}$ (h)	12.68 ± 1.84*	11.83 ± 1.56*	10.10 ± 1.82*
V_d (L)	1.2573 ± 0.03	0.7305 ± 0.05	0.5223 ± 0.0045
$AUC_{0\rightarrow\infty}$ ($ng{\cdot}h{\cdot}ml^{-1}$)	21007.19 ± 1543	34197.55 ± 1383	41113.41 ± 1729
MRT (h)	9.62 ± 1.32	9.51 ± 1.56	9.06 ± 0.72
Cl ($ml{\cdot}h^{-1}$)	0.07 ± 0.002	0.04 ± 0.001	0.04 ± 0.001
TCR (L/h)	0.07 ± 0.004	0.04 ± 0.004	0.04 ± 0.002

Mean ± S.D., n = 6, * $p<0.05$ (ANOVA test)

5.2.2. Pharmacokinetic study of Cilostazol-DM-β-CD inclusion complex (1:3) prepared by co-precipitation method, Marketed formulation (Platoz-50) and Pure Cilostazol.

5.2.2.1. Experimental Design

The pharmacokinetic study was performed in New Zealand rabbits weighing 1.0 to 1.5 kg. The animals were fasted over night prior to the experiment but had free access to water. The conventional tablet(Platoz-50) powder equivalent to 7.0 mg. of Cilostazol, Cilostazol-DM-β-CD inclusion complex in the ratio of 1:3 (equivalent to 7.0 mg. of Cilostazol) and pure Cilostazol (7.0 mg.) were taken and mixed with 1 ml of 0.5 % of sodium -carboxy methyl cellulose solution (Na-CMC). Prepared suspensions were administered orally to the rabbits as per the dose calculation method mention in section 5.2.1.1.There were three groups containing eight rabbits in each group as, two rabbits for placebo, two rabbits for pure Cilostazol, two rabbits for conventional tablet and two rabbits for Cilostazol-DM-β-CD inclusion complex were selected. Blood samples were collected from the marginal ear vein at 1, 2, 3, 4, 6, 12, 18 and 24 hrs after the administration. The heparinised blood samples were immediately centrifuged at 40000 rpm for 15 minutes and separated plasma was stored at -20 °C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method described in section 3.1.3.and the drug plasma concentration values were determined from the calibration curve. The average drug plasma concentration of pure Cilostazol, conventional tablet and Cilostazol-DM-β-CD inclusion complex are shown in Table 5.2.2.1. The average plasma drug concentration versus time profiles for all three formulations are given in Figure. 5.2.2.1 and the different pharmacokinetic parameters were calculated by using Wagner-Nelson method (Table 5.2.2.2.).

Table 5.2.2.1. Average plasma Cilostazol concentration after administration of Pure Cilostazol, conventional tablet and Cilostazol-DM-β-CD inclusion complex (1:3) in rabbits.

Sr. No	Time (hour)	Plasma concentration in ng/ml ± SD		
		Pure Cilostazol	Conventional Tablet (Platoz-50)	Cilostazol-DM-β-CD inclusion Complex
1	0	00.00 ± 0.86	00.00 ± 0.27	00.00 ± 1.63
2	1	680.24 ± 3.42	945.15 ± 6.23	1846.88 ± 3.19
3	2	921.48 ± 5.86	1486.64 ± 9.06	3334.25 ± 4.82
4	3	1150.6 ± 6.85	2126.48 ± 8.67	4734.88 ± 13.07
5	4	1021.11 ± 8.07	1735.91 ± 8.37	4127.72 ± 10.93
6	6	836.34 ± 7.44	1478.45 ± 7.25	2915.15 ± 6.26
7	12	601.44 ± 5.91	988.56 ± 5.14	1835.45 ± 5.47
8	18	450.56 ± 4.38	736.28 ± 4.29	1048.55 ± 4.99
9	24	328.37 ± 1.49	529.82 ± 2.58	548.45 ± 3.31

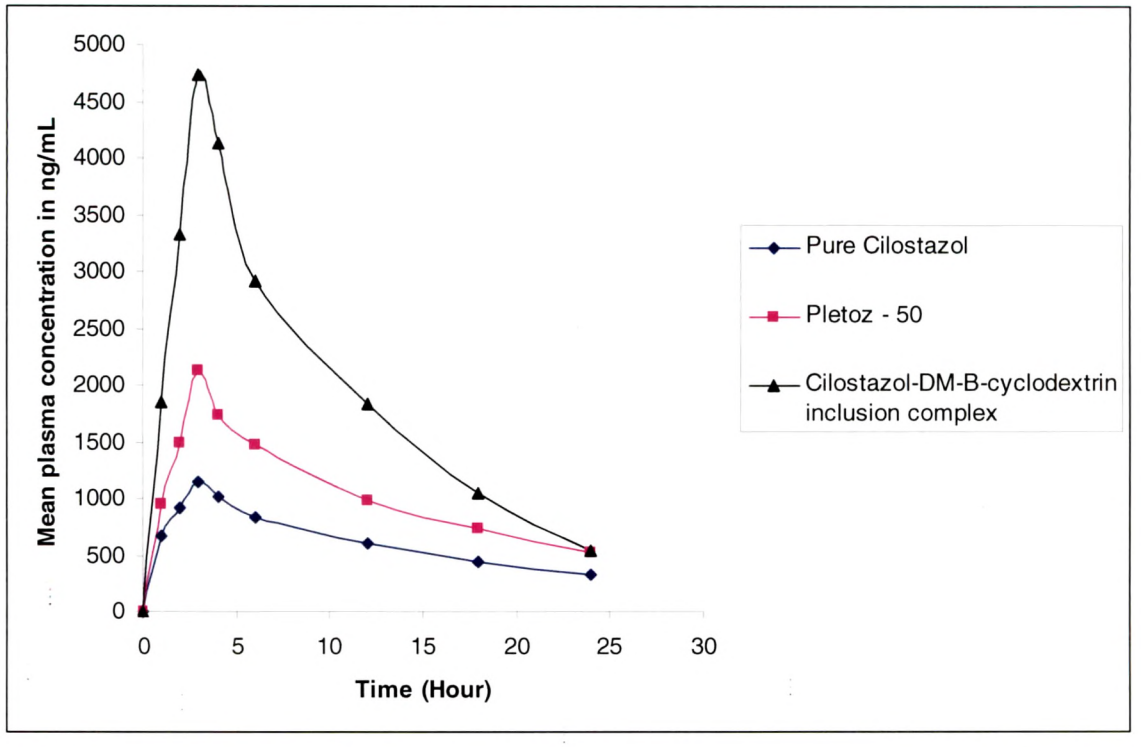

Figure 5.2.2.1. Comparison of pharmacokinetic profiles of Pure Cilostazol, Conventional tablet and Cilostazol-DM-β-CD inclusion complex.

Table 5.2.2.2. Different pharmacokinetic parameters calculated by Wagner-Nelson method.

Parameters #	Pure Cilostazol	Marketed formulation (Platoz-50)	Cilostazol-DM-β-CD inclusion Complex
C_{max} (ng/ml)	1150.96 ± 101*	2126.48 ± 185*	4734.88 ± 252*
t_{max} (h)	3.90 ± 1.32	4.00 ± 0.93	3.50 ± 0.66
k_a (h^{-1})	0.77 ± 0.006	0.63 ± 0.003	0.71 ± 0.001
k_{el} (h^{-1})	0.05 ± 0.002	0.06 ± 0.0032	0.09 ± 0.0022
$T_{1/2}$ (h)	12.68 ± 1.84*	11.83 ± 1.56*	7.46 ± 1.58*
V_d (L)	1.2573 ± 0.03	0.7305 ± 0.05	0.3020 ± 0.007
$AUC_{0\rightarrow\infty}$ ($ng{\cdot}h{\cdot}ml^{-1}$)	21007.19 ± 1543	34197.55 ± 1383	52625.59 ± 1482
MRT (h)	9.62 ± 1.32	9.51 ± 1.56	8.57 ± 2.01
Cl ($ml{\cdot}h^{-1}$)	0.07 ± 0.002	0.04 ± 0.001	0.03 ± 0.0023
TCR (L/h)	0.07 ± 0.004	0.04 ± 0.004	0.03 ± 0.0015

Mean ± S.D., n = 6, *p<0.05 (ANOVA test)

5.2.3. Pharmacokinetic study for Tadalafil oral Microemulsion (TME 3), Marketed formulation (Tadora-20) and Pure Tadalafil.

5.2.3.1.CALCULATION OF DOSE OF DRUG IN RABBITS

The dose of the drug in the rabbits was calculated, depending on the weight of the rabbits in mg/kg. By the following formula[7,8]:

HED (Human Equivalent Dose) for rabbit = 0.07 × Human dose of the drug

Where the rabbit weight was considered as 1.5 Kg.

The maximum dose of Tadalafil that can be given to human in single day is 20 mg. According to the above formula, the dose for the rabbits is calculated to be 0.93 mg/kg. In this study, the dose given to the rabbits is 1.4 mg/1.5Kg of rabbit weight.

5.2.3.2. Experimental Design

The pharmacokinetic study was performed in New Zealand rabbits weighing 1.0 to 1.5 kg. The animals were fasted over night prior to the experiment but had free access to water. The conventional tablet (tadora-20) powder equivalent to 1.4 mg. of Tadalafil and pure Tadalafil (1.4 mg.) were taken and mixed with 1 ml of 0.5 % of sodium-carboxy methyl cellulose solution (Na-CMC). Oral microemulsion containing 1.4 mg/ml of Tadalafil was prepared and given orally to the rabbits as per the dose calculation method mentioned in section 5.2.3.1. There were three groups containing eight rabbits in each group as, two rabbits for placebo, two rabbits for pure Tadalafil, two rabbits for conventional tablet and two rabbits for oral microemulsion containing Tadalafil were selected. Blood samples were collected from the marginal ear vein at 1, 2, 3,4, 6, 12, 18, 24, 30 and 36 hrs after the administration. The heparinised blood samples were immediately centrifuged at 40000 rpm for 15 minutes and separated plasma was stored at -20 °C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method described in section 4.1.3.and the drug plasma concentration values were determined from the calibration curve. The average drug plasma concentration of pure Tadalafil, conventional tablet and oral microemulsion are shown in Table 5.2.3.1. The average plasma drug concentration versus time profiles for all three formulations are given in Figure. 5.2.3.1 and the different pharmacokinetic parameters were calculated by using Wagner-Nelson method (Table 5.2.3.2.).

Table 5.2.3.1. Average plasma Tadalafil concentration after administration of pure Tadalafil, Conventional tablet and Oral microemulsion of Tadalafil (TME 3) in rabbits.

Sr. No	Time (hour)	Plasma concentration in ng/ml ± SD		
		Pure Drug	Conventional Tablet	Oral Microemulsion
1	0	00.00 ± 1.24	00.00 ± 1.54	00.00 ± 0.66
2	1	112.00 ± 6.14	82.00 ± 6.35	357.68 ± 5.37
3	2	258.00 ± 8.58	150.60 ± 7.82	737.95 ± 7.48
4	3	290.55 ±9.71	323.28 ± 6.88	1129.5 ± 6.83
5	4	376.00 ± 8.88	484.03 ± 7.27	1088.00 ± 12.15
6	6	352.86 ± 6.94	388.66 ±6.92	952.72 ±14.03
7	12	261.57 ± 6.85	305.19 ± 5.45	720.32 ±11.7
8	18	206.02 ± 5.14	224.48 ± 6.33	556.00 ± 9.55
9	24	165.51 ± 3.81	185.70 ± 4.25	335.00 ± 6.35
10	30	129.21 ± 1.36	143.96 ± 3.57	214.63 ± 7.97
11	36	103.00 ±	112.80 ± 2.88	121.23 ± 4.33

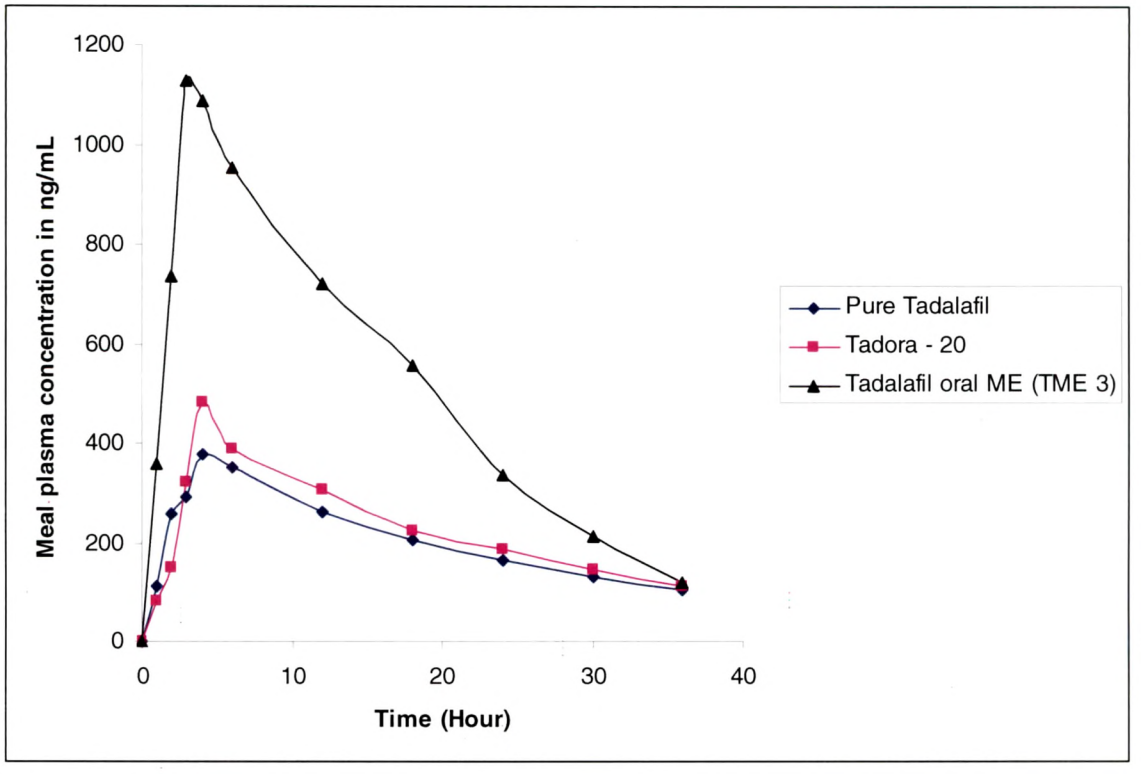

Figure 5.2.3.1. Comparison of pharmacokinetic profiles of Pure Tadalafil, Conventional tablet and Oral microemulsion of Tadalafil (TME 3).

Table 5.2.3.2. Different pharmacokinetic parameters calculated by Wagner-Nelson method.

Parameters #	Pure Drug	Marketed formulation (Tadora – 20)	Microemulsion
C_{max} (ng/ml)	376.00 ± 79*	484.03 ± 85*	1129.50 ±315*
t_{max} (h)	4.00 ± 1.00	4.0 ± 0.95	3.10 ± 1.08
k_a (h^{-1})	1.60 ± 0.04	0.49± 0.036	0.51 ± 0.02
k_{el} (h^{-1})	0.04 ± 0.007	0.04 ± 0.008	0.07 ± 0.01
$t_{1/2}$ (h)	16.99 ± 2.01*	16.96 ± 1.86*	9.42 ± 1.63*
V_d (L)	4.3459 ± 0.79	3.1295 ± 0.84	0.8820 ±0.36
$AUC_{0\rightarrow\infty}$ ($ng{\cdot}h{\cdot}ml^{-1}$)	10203.31 ± 1050	11125.24.69 ± 1628	21733.55 ± 3256
MRT (h)	14.34± 1.31	14.53± 1.38	12.24 ± 1.26
Cl ($ml{\cdot}h^{-1}$)	0.18 ± 0.066	0.13 ± 0.051	0.09 ± 0.003
TCR (L/h)	0.18	0.13	0.09

Mean ± S.D., n = 6, *p<0.05 (ANOVA test)

5.2.4. Pharmacokinetic study of Tadalafil-β-CD inclusion complex (1:3) prepared by kneading method, Marketed formulation (Tadora-20) and Pure Tadalafil.

5.2.4.1. Experimental Design

The pharmacokinetic study was performed in New Zealand rabbits weighing 1.0 to 1.5 kg. The animals were fasted over night prior to the experiment but had free access to water. The conventional tablet(Tadora-20) powder equivalent to 1.4 mg. of Tadalafil, Tadalafil-β-CD inclusion complex in the ratio of 1:3 (equivalent to 1.4 mg. of Tadalafil) and pure Tadalafil (1.4 mg.) were taken and mixed with 1 ml of 0.5 % of sodium -carboxy methyl cellulose solution(Na-CMC). Prepared suspensions were administered orally to the rabbits as per the dose calculation method mention in section 5.2.3.1.

There were three groups containing eight rabbits in each group as, two rabbits for placebo, two rabbits for pure Tadalafil, two rabbits for conventional tablet and two rabbits for Tadalafil-β-CD inclusion complex were selected. Blood samples were collected from the marginal ear vein at 1, 2, 3, 4, 6, 12, 18, 24, 30 and 36 hrs after the administration. The heparinised blood samples were immediately centrifuged at 40000 rpm for 15 minutes and separated plasma was stored at -20 °C.

Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method described in section 4.1.3.and the drug plasma concentration values were determined from the calibration curve. The average drug plasma concentration of pure Tadalafil, conventional tablet and Tadalafil-β-CD inclusion complex are shown in Table 5.2.4.1. The average plasma drug concentration versus time profiles for all three formulations are given in Figure. 5.2.4.1 and the different pharmacokinetic parameters were calculated by using Wagner-Nelson method (Table 5.2.4.2.).

Table 5.2.4.1. Average plasma Tadalafil concentration after administration of Pure drug, conventional tablet and Tadalafil-β-CD inclusion complex in rabbits.

Sr. No	Time (hour)	Plasma concentration in ng/ml ± SD		
		Pure Drug	Conventional Tablet	Tadalafil-β-CD inclusion Complex
1	0	00.00 ± 1.24	00.00 ± 1.54	00.00 ± 1.25
2	1	112.00 ± 6.14	82.00 ± 6.35	115.00 ± 3.76
3	2	258.00 ± 8.58	150.60 ± 7.82	412.00 ± 9.56
4	3	290.55 ±9.71	323.28 ± 6.88	684.82 ±11.21
5	4	376.00 ± 8.88	484.03 ± 7.27	551.58 ± 7.34
6	6	352.86 ± 6.94	388.66 ±6.92	475.00 ± 6.55
7	12	261.57 ± 6.85	305.19 ± 5.45	371.21 ± 5.94
8	18	206.02 ± 5.14	224.48 ± 6.33	286.00 ± 3.83
9	24	165.51 ± 3.81	185.70 ± 4.25	205.32 ± 5.64
10	30	129.21 ± 1.36	143.96 ± 3.57	150.71 ± 6.92
11	36	103.00 ±	112.80 ± 2.88	118.12 ± 3.33

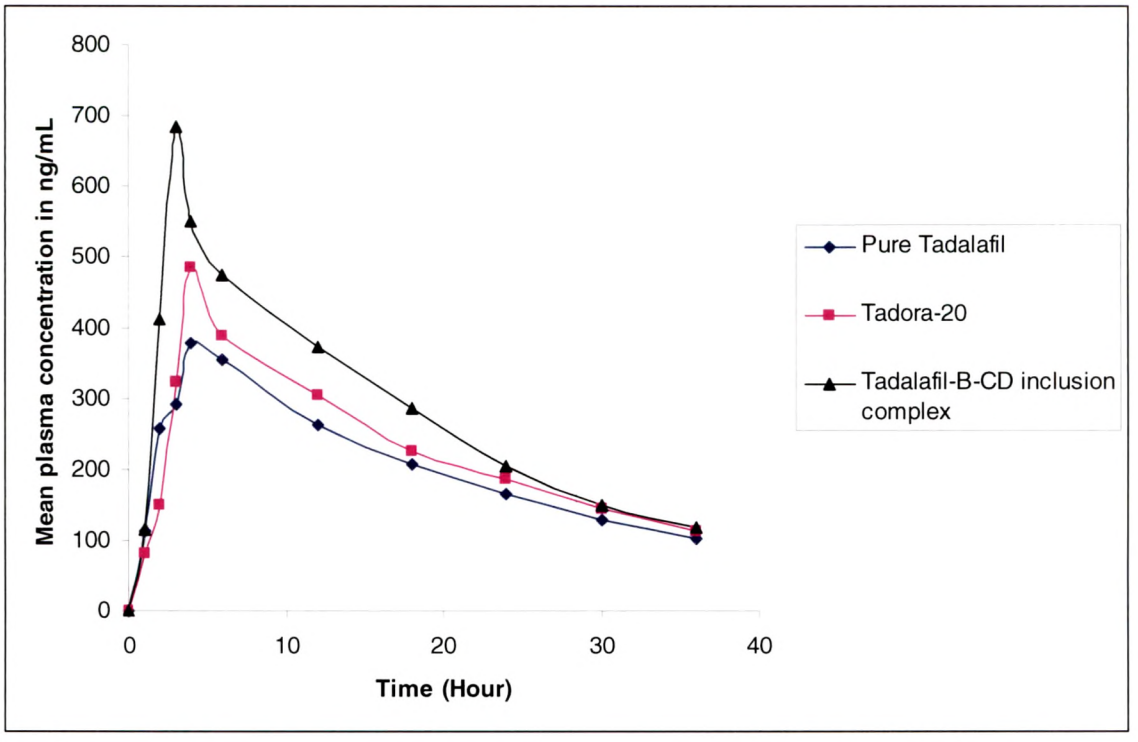

Figure 5.2.4.1. Comparison of pharmacokinetic profiles of Pure Tadalafil, Conventional tablet and Tadalafil-β-CD inclusion complex.

Table 5.2.4.2. Different pharmacokinetic parameters calculated by Wagner-Nelson method.

Parameters #	Pure Drug	Marketed formulation (Tadora – 20)	Tadalafil-β-CD inclusion Complex
C_{max} (ng/ml)	376.00 ± 79*	484.03 ± 85*	684.82 ± 112*
t_{max} (h)	4.00 ± 1.00	4.0 ± 0.95	4.00 ± 1.15
k_a (h^{-1})	1.60 ± 0.04	0.49± 0.036	0.38 ±0.055
k_{el} (h^{-1})	0.04 ± 0.007	0.04 ± 0.008	0.05 ± 0.003
$t_{1/2}$ (h)	16.99 ± 2.01*	16.96 ± 1.86*	11.23 ± 1.26*
V_d (L)	4.3459 ± 0.79	3.1295 ± 0.84	3.3348 ±0.69
$AUC_{0\rightarrow\infty}$ ($ng{\cdot}h{\cdot}ml^{-1}$)	10203.31 ± 1050	11125.24.69 ± 1628	13086.09 ±2120
MRT (h)	14.34± 1.31	14.53± 1.38	13.54 ± 1.12
Cl ($ml{\cdot}h^{-1}$)	0.18 ± 0.066	0.13 ± 0.051	0.14 ± 0.059
TCR (L/h)	0.18	0.13	0.16

Mean ± S.D., n = 6, *p<0.05 (ANOVA test)

5.3. Result and Discussion

In Vivo absorption study was performed to evaluate the oral absorption rate in to the living cells, tissues and organism. The study was carried out in male New Zealand rabbits. As per the results obtained in previous studies, CD inclusion and oral microemulsion dramatically increased the solubility, *in vitro* dissolution and permeability of both the drugs, Cilostazol and Tadalafil.

The *in vitro* permeability study of Cilostazol MEs showed that CME 2 formulation has a higher diffusion rate compared to pure Cilostazol and marketed formulation (Pletoz - 50) and hence it was selected for *in vivo* absorption study. The plasma concentration Vs time profiles after oral administration of Pure Cilostazol, conventional tablet (Platoz-50) and CME 2 are shown in Figure 5.2.1.1.The pharmacokinetic parameters for the same are described in Table 5.2.1.2.The results show that the **C_{max} of CME 2 was 2.65 fold higher than pure Cilostazol and 1.43 fold higher than marketed formulation (Pletoz – 50)**.The value of $T_{1/2}$ was also decreased in CME 2 (10.10 Hr.) compared to that of pure Cilostazol (12.68) and Platoz-50(11.83).Decreased value of $T_{1/2}$ for CME 2 indicated that the *in vivo* absorption was rapid and higher compared to pure Cilostazol and Platoz-50.

From the *in vitro* dissolution study of all Cilostazol-CDs inclusion complexes and Cilostazol-CDs physical mixtures, Cilostazol-DM-β-CD inclusion complex in the ratio of 1:3 prepared by co-precipitation method showed higher dissolution rate compared to all Cilostazol-CDs physical mixtures, marketed formulation (Platoz-50) and Pure Cilostazol. Therefore, Cilostazol-DM-β-CD inclusion complex (1:3) prepared by co-precipitation method was selected for *in vivo* absorption study. The plasma concentration Vs time profiles after oral administration of Pure Cilostazol, conventional tablet (Platoz-50) and Cilostazol-DM-β-CD inclusion complex are shown in Figure 5.2.2.1. The pharmacokinetic parameters for the same are described in Table 5.2.2.2. The results showed that the **C_{max} of Cilostazol-DM-β-CD inclusion complex was 4.11 fold higher than pure Cilostazol and 2.23 fold higher than marketed formulation (Pletoz – 50).** The value of $T_{1/2}$ was also decreased in Cilostazol-DM-β-CD inclusion complex (7.46 Hr.) compared with pure Cilostazol (12.68) and Platoz-50(11.83). A Sharp reduction in $T_{1/2}$ value indicated faster *in vivo* absorption compared to pure Cilostazol and Platoz-50.

The *in vitro* permeability study of Tadalafil ME showed that TME 3 formulation has a higher diffusion rate compared to that of pure Tadalafil and marketed formulation (Tadora – 20) and hence it was selected for *in vivo* absorption study. The plasma concentration Vs time profiles after oral administration of Pure Tadalafil, conventional tablet (Tadora-20) and TME 3 are shown in Figure 5.2.3.1.The pharmacokinetic parameters for the same are described in Table 5.2.3.2. The results show that the **C_{max} of TME 3 was 3.00 fold higher than pure Tadalafil and 2.33 fold higher than marketed formulation (Tadora – 20).**The value of $T_{1/2}$ was also decreased in TME 3 (9.42 Hr.) compared to that of pure Tadalafil (16.99) and Tadora-20(16.96). Drastically reduced $T_{1/2}$ value indicated faster *in vivo* absorption compared to pure Tadalafil and Tadora-20.

The *in vitro* dissolution study of all Tadalafil-β-CDs inclusion complexes and Tadalafil-β-CDs physical mixtures suggested that Tadalafil -β-CD inclusion complex in the ratio of 1:3 prepared by kneading method has higher dissolution rate compared to Tadalafil-CDs physical mixtures, Tadalafil-β-CDs inclusion complexes, marketed formulation (Tadora-20) and pure Tadalafil.Therefore, Tadalafil-β-CD inclusion complex (1:3) prepared by kneading method was selected for *in vivo* absorption study. The plasma concentration Vs time profiles after oral administration of pure Tadalafil, conventional tablet (Tadora-20) and Tadalafil -β-CD inclusion complex are shown in Figure 5.2.4.1. The pharmacokinetic parameters for the same are described in Table 5.2.4.2. The results of the study show that the **C_{max} of Tadalafil-β-CD inclusion complex was 1.81 fold higher than pure Cilostazol and 1.41 fold higher than marketed formulation (Tadora – 20).** The value of $T_{1/2}$ was also decreased in Tadalafil -β-CD inclusion complex (11.23 Hr.) compared to that of Tadalafil (16.99) and Tadora-20 (16.96). Decreased value of $T_{1/2}$ for Tadalafil-β-CD inclusion complex indicated that the *in vivo* absorption was rapid and higher compared to pure Tadalafil and Tadora-20.

Amongst all formulations (MEs and Inclusion complexes) of Cilostazol and Tadalafil, Cilastazol-DM-β-CD (1:3) prepared by Co-precipitation method and TME 3 microemulsion having S:CoS ratio (3:1) showed highest absorption rate.

Finally, the enhancement of oral absorption rate may be attributed to (1) the large specific surface area of the microemulsion droplets(droplet size less that 90 nm) and CD inclusion complex (2) improved permeation because of the presence of surfactant, which reduced the interfacial tension, (3) the stability of the microemulsion in the gastrointestinal

tract,(4) improved dissolution because of the hydrophilic exterior surface of CD and (5) the stability of inclusion complex in the gastrointestinal tract.

5.4. References

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