CHAPTER IV

IN VIVO EVALUATION

Dissolution rate (*in vitro*) tests are not necessarily a measure of therapeutic efficacy or safety of a drug or its dosage form. It therefore, becomes important to correlate the *in vitro* data with *in vivo* data in order to get a clear picture of effectiveness or safety of the drug or its dosage form.

Several workers have reviewed the *in vivo* methods for evaluation of prolonged release dosage forms (1-7). The pharmacological response in animals has often been used as a method for evaluation. Depending upon the drug to be tested, experiments are designed to measure a specific response to be produced by the drug (8).

The clinical response or measurement of duration of therapeutic response can also be used for evaluation of prolonged action formulations. These methods are used for drugs which are very potent and cannot be quantitatively determined in body fluids, but these studies often lack objectivity (9).

The determination of blood levels of drug affords another method for evaluation of prolonged release products of certain drugs for which there exists a relationship between blood level and clinical response (10). The measurement of drug concentration provides a means of determining the time of onset and duration of action, if

minimum effective concentration is known. The blood level analysis have been used for the evaluation of the products of several drugs such as prednisolone (11), caffeine (12), sulphaethylthiadiazole (13) and penicillin (14).

Urinary excretion rates have been used to evaluate duration of drug action, since in certain cases there is a direct relationship between excretion rate and amount of drug in the blood. Several drugs including aspirin (15), penicillin (16), tetracycline (17), trimeprazine (18) and phenylpropanolamine (19) have been studied by the urinary excretion technique. The technique can be applied to only those drugs which are excreted either unchanged or as therapeutically inactive metabolites (20).

Recently radioactive isotopes have been recommended as tracers in animal and human studies to study drug utilization (21-23). The nonavailability of radioactive drugs has been a limiting factor in not too wide a use of this technique.

In the present investigation, controlled release products of tetracycline hydrochloride and hydralazine hydrochloride were evaluated in normal human volunteers and dogs respectively employing a crossover design.

A. TETRACYCLINE HYDROCHLORIDE

Procedure

Four healthy male human volunteers weighing 55 to 75 kg aging 26-34 years were selected for *in vivo* evaluation of conventional and controlled release capsules and tablets of tetracycline hydrochloride. Experiment was carried out in a crossover design allowing one week washout period in between. In the morning, second urine was collected as blank and a capsule/tablet was administered orally with about 200 ml of water. Urine samples were collected at 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 hours after administration of the sample. The urine volume was measured and recorded after each collection, aliquots were frozen till assayed. Samples were assayed as per method described in Chapter II (Page 45), within 24 hours of collection. Results are recorded in Tables 4-1 to 4-3 and shown graphically in Figures 4-1 and 4-2.

Statistical Analysis

Average values of the urinary excretion rates with standard errors, at midpoint of urine collection time were calculated from results of cumulative amount excreted of tetracycline hydrochloride after oral administration of each formulation (24). Average tetracycline serum levels

TABLE 4-1 : COMPARATIVE URINARY EXCRETION RATES AND TOTAL AMOUNT EXCRETED OF TETRACYCLINE HYDROCHLORIDE AFTER ORAL ADMINISTRATION OF CONTROLLED RELEASE AND CONVENTIONAL PRODUCTS

Time (Midpoint of urine collection time) (hrs.)	MEAN EXCRETION RATES (mg hr^{-1})									
	a		b		c		d		e	
	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.
0.5	5.74	0.325	2.09	0.370	4.41	0.290	2.26	0.310	2.48	0.305
1.5	6.74	0.345	3.94	0.395	6.67	0.340	2.87	0.280	3.20	0.355
3.0	7.34	0.375	5.76	0.313	8.33	0.395	3.73	0.355	5.78	0.360
5.0	6.55	0.405	2.30	0.365	5.91	0.360	3.63	0.260	3.46	0.280
7.0	2.80	0.160	1.37	0.390	7.22	0.260	3.75	0.315	2.86	0.240
10.0	1.96	0.040	0.73	0.075	5.08	0.280	2.50	0.130	2.38	0.195
15.0	0.47	0.005	0.38	0.055	1.80	0.090	0.98	0.090	1.06	0.095
21.0	0.08	0.010	0.06	0.005	0.92	0.065	0.13	0.030	0.24	0.045
30.0	-	-	-	-	0.30	0.030	-	-	-	-
42.0	-	-	-	-	-	-	-	-	-	-
Total amount of Tetracycline excreted in 48 hrs (mg)	129.79		68.13		166.49		81.32		87.92	

Key
a 500 mg conventional capsule
b 250 mg conventional capsule
c 500 mg controlled release capsule
d 250 mg controlled release capsule
e 250 mg controlled release tablet

S.E. = Standard Error

FIG.·4-1: COMPARATIVE URINARAY EXCRETION RATES OF TETRACYCLINE HYDROCHLORIDE AFTER ORAL ADMINISTRATION OF CONTROLLED RELEASE AND CONVENTIONAL PRODUCTS.

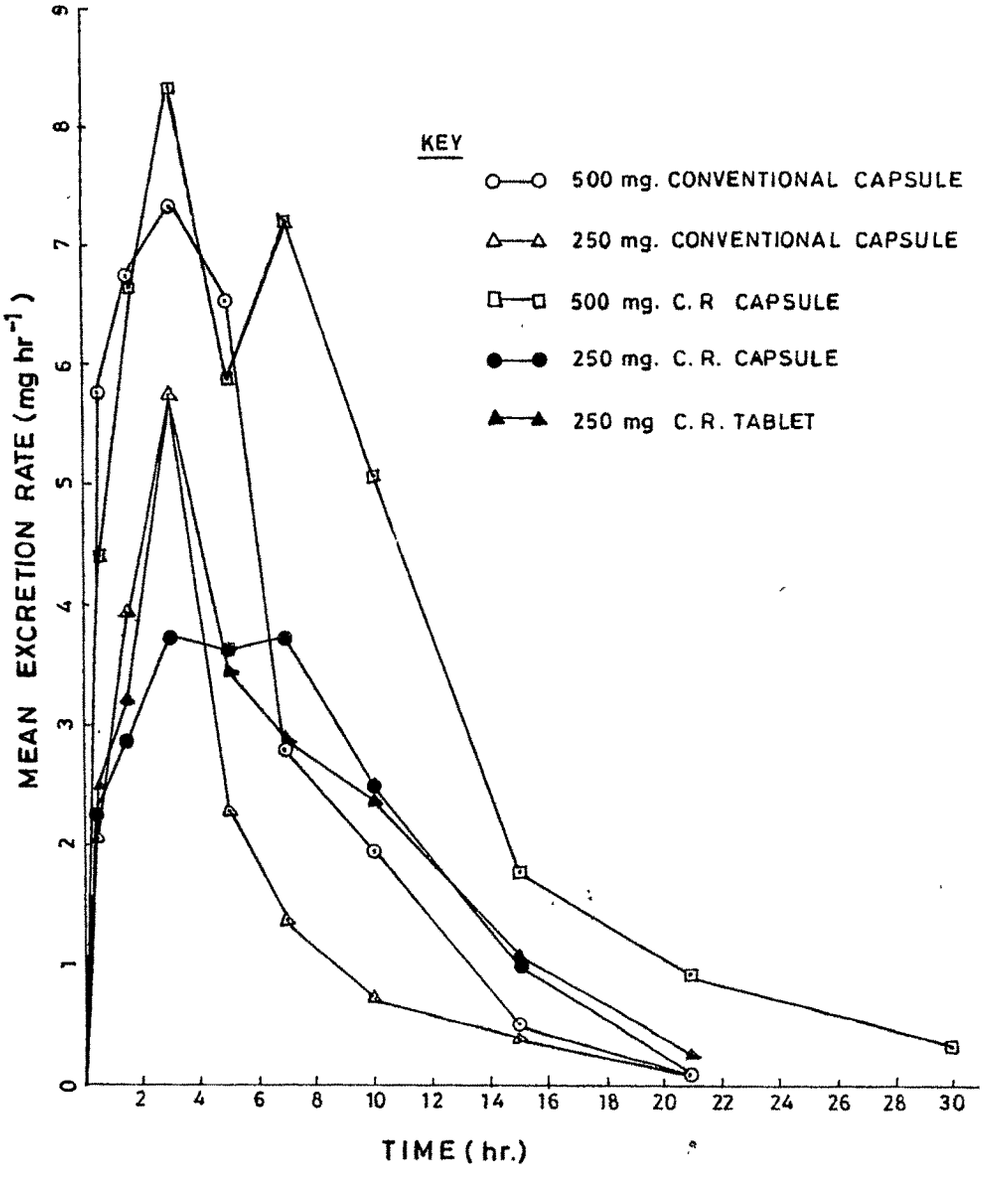


TABLE 4-2 : PREDICTED SERUM LEVELS OF TETRACYCLINE HYDROCHLORIDE AFTER ORAL ADMINISTRATION OF CONVENTIONAL AND CONTROLLED RELEASE PRODUCTS AND THEIR COMPARISON

Time (Mid-point of urine collection time (Hrs.)	Mean serum levels ($\mu g\ ml^{-1}$)										Mean serum levels compared by paired t-test (level of significance)		
	Mean[a]	±S.E.	Mean[b]	±S.E.	Mean[c]	±S.E.	Mean[d]	±S.E.	Mean[e]	±S.E.	a vs c	b vs d	b vs c
0.5	1.14	0.070	0.42	0.080	0.88	0.065	0.46	0.070	0.50	0.070	S	NS	S
1.5	1.34	0.075	0.80	0.080	1.32	0.075	0.58	0.060	0.64	0.075	NS	S	S
3.0	1.46	0.080	1.14	0.070	1.66	0.080	0.74	0.075	1.14	0.075	S	S	NS
5.0	1.30	0.090	0.46	0.080	1.16	0.075	0.72	0.060	0.70	0.060	S	S	S
7.0	0.56	0.040	0.28	0.080	1.42	0.060	0.74	0.070	0.58	0.050	S	S	S
10.0	0.40	0.010	0.16	0.020	1.02	0.060	0.52	0.030	0.48	0.045	S	S	S
15.0	0.10	0.020	0.08	0.020	0.36	0.020	0.22	0.020	0.22	0.030	S	S	S
21.0	0.02	0.005	0.02	0.005	0.18	0.020	0.04	0.010	0.06	0.010	S	NS	NS
30.0	-	-	-	-	0.08	0.010	-	-	-	-	S	-	-
42.0	-	-	-	-	-	-	-	-	-	-	-	-	-

Key
- a 500 mg conventional capsule
- b 250 mg conventional capsule
- c 500 mg controlled release capsule
- d 250 mg controlled release capsule
- e 250 mg controlled release tablet

S.E. = Standard Error.
S - Significant at the 0.05 level.
NS - Not Significant.

FIG.·4-2: PREDICTED SERUM LEVELS OF TETRACYCLINE AFTER ORAL ADMINISTRATION OF CONTROLLED RELEASE AND CONVENTIONAL PRODUCTS.

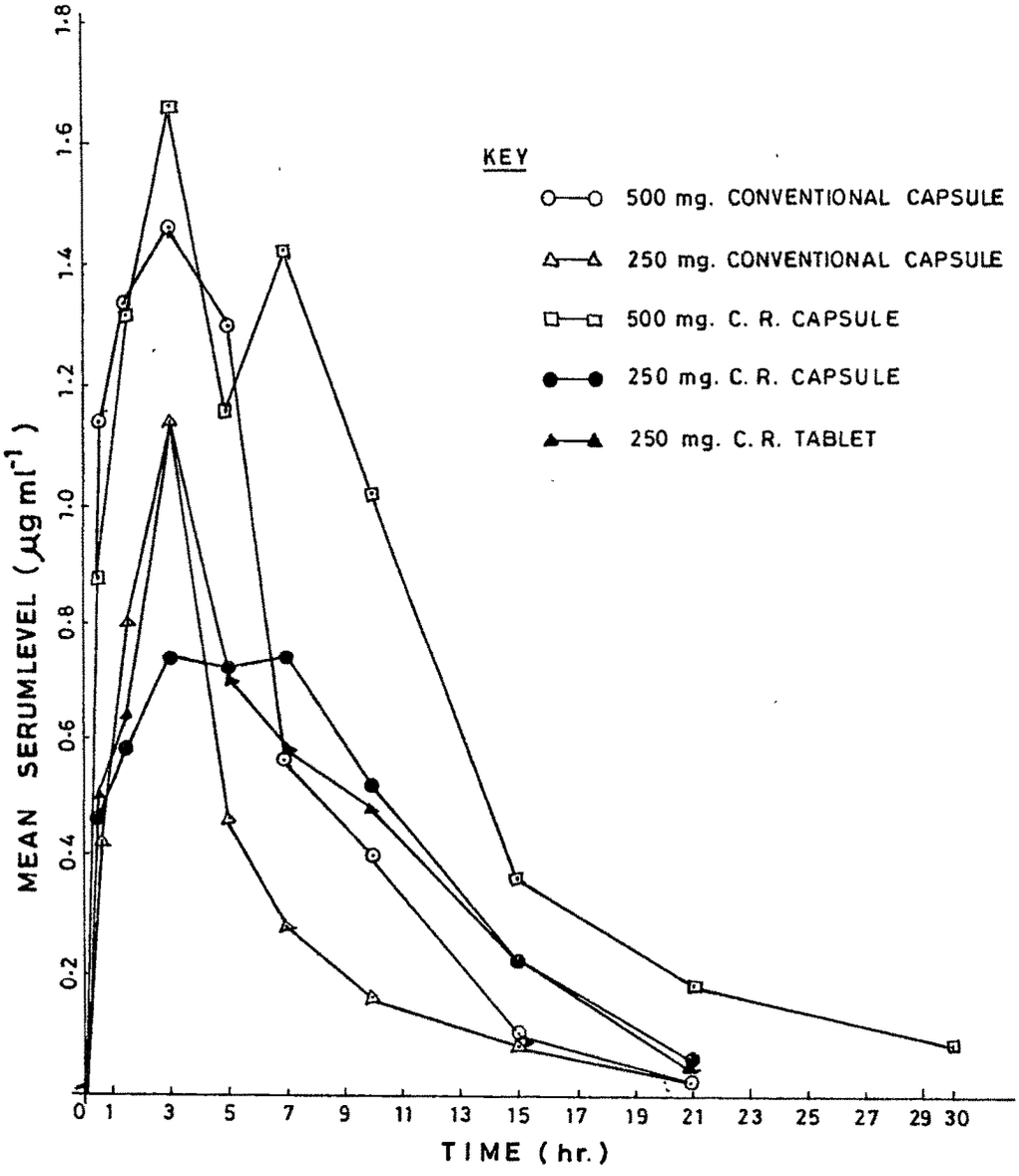


TABLE 4-3 : PHARMACOKINETIC PARAMETERS AFTER ORAL ADMINISTRATION OF CONVENTIONAL AND CONTROLLED RELEASE PRODUCTS OF TETRACYCLINE HYDROCHLORIDE

Product	a		b		c		d		e	
Pharmaco-kinetic parameter	Mean	± S.E.	Mean	±.S.E.	Mean	± S.E.	Mean	± S.E.	Mean	± S.E.
T_{max} (Hrs)	3.00	0.250	3.00	0.250	3.00	0.250	3.00	0.250	3.00	0.250
C_{max} (µg/ml)	1.46	0.080	1.14	0.070	1.66	0.080	0.74	0.075	1.14	0.075
Peak to trough ratio	3.65	0.805	7.13	1.512	1.63	0.182	1.42	0.243	2.38	0.413
Bioavailability (%)	-		-		128.28		119.36		129.05	
AUC_{0-30} (µg hr ml^{-1})	11.29	0.730	5.80	0.845	16.36	1.330	9.06	0.810	9.27	0.895

Key

a 500 mg conventional capsule

b 250 mg conventional capsule

c 500 mg controlled release capsule

d 250 mg controlled release capsule

e 250 mg controlled release tablet

S.E. = Standard Error.

T_{max} : Time required to achieve peak serum concentration.

C_{max} : Peak serum concentration.

Peak to trough ratio : C_{max} / serum concentration at 10 hrs.

Bioavailability: $\frac{\text{Amount excreted by C.R. product}}{\text{Amount excreted by conventional product}}$

AUC_{0-30} Area under serum level curve (0 to 30 hrs)

with standard errors were predicted from the data of urinary excretion rates (25). The average serum levels after administration of conventional and controlled release products were compared by paired t-test. A similar analysis was performed on the areas under the serum concentration curves.

RESULTS AND DISCUSSION

Urinary Excretion Rates :

The urinary excretion rates of tetracycline hydrochloride at the midpoint of urine collection time after oral administration of each of the products (Table 4-1) were computed from the data of cumulative amount excreted of tetracycline hydrochloride. Bioavailability of controlled release products were calculated from total amount excreted of tetracycline hydrochloride in 48 hrs (Table 4-1) after administration of controlled release and conventional products (26) and results are shown in Table 4-3.

Serum Levels :

Approximate serum levels of tetracycline hydrochloride at the midpoint of urine collection time as shown in Table 4-2, were predicted based on relationship between urinary excretion rates and serum levels reported by Barr *et al.* (25).

Analysis of these data at time points by paired t-test revealed the following significant differences ($P < 0.05$) :

1) The mean peak tetracycline serum level was significantly higher in case of 500 mg controlled release capsule than 500 mg conventional capsule. However, C_{max} values with 250 mg controlled release tablet and capsule were same and lower respectively as compared to C_{max} attained with 250 mg conventional capsule.

2) The mean tetracycline serum levels, attained between 3 to 30 hrs with 500 mg controlled release capsule were higher than those achieved with the 500 mg conventional capsule.

3) The mean tetracycline serum levels between 5-21 hrs were higher with the 250 mg controlled release capsule and tablet when compared with the serum levels obtained after administration of 250 mg conventional capsule.

4) Peak to trough ratio was calculated for both conventional and controlled release products from C_{max} values and serum level at 10 hrs. Peak to trough ratio was higher with conventional products.

5) Areas under the serum level curves after administration of each product were calculated using trapezoidol rule (26). Controlled release products have greater areas under the curves as compared to conventional product.

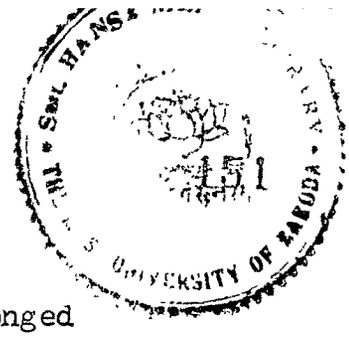
These studies have shown that controlled release products have significantly higher bioavailability in comparison to conventional product. As an amphoteric substance, tetracycline forms salts with acids as well as with bases. With hydrochloric acid it forms a hydrochloride, the solutions of which are strongly subjected to hydrolysis in a neutral medium. Tetracycline bases which precipitate above pH 3 are very difficultly soluble (27). Therefore, absorption of tetracycline hydrochloride is limited to small area of GI tract. In case of controlled release products of present investigation, as soon as they come to a region with a pH value of 3 or more, such as upon leaving stomach; the medium is influenced by the organic acid (succinic acid of the product) in such a way that the pH value of the surrounding fluid never exceeds a pH value which would permit hydrolysis of the tetracycline and thereby precipitation of the free base (28). This makes tetracycline hydrochloride available throughout the GI tract from controlled release products.

Higher mean tetracycline peak serum levels are achieved with 500 mg and 250 mg controlled release capsule and tablet as compared to 500 mg and 250 mg conventional capsules respectively, and these peaks are achieved in same time. 250 mg controlled release capsule has shown comparatively lower mean tetracycline serum peak level

as compared to 250 mg conventional capsule. However, the mean tetracycline serum levels attained between 3 to 30 hrs and 5 to 21 hrs with 500 mg and 250 mg controlled release capsules and tablet were higher than those achieved with the 500 mg and 250 mg conventional capsules respectively. Smaller is the peak to trough ratio better is the sustained action. Controlled release products studied have shown comparatively much smaller peak to trough ratio.

The areas under the curves are significantly higher for the controlled release products in comparison with those achieved by conventional tetracycline hydrochloride in single dose. This may be due to comparatively faster changes in serum concentrations of the drug following the administration of the conventional capsule.

From these studies, the controlled release products appear to be promising with regard to making medication simpler to the patients and to reduce fluctuation in tetracycline levels throughout the therapy. Controlled release products have shown comparatively better bio-availability. Further, the incidence of side effects should be established through clinical trials. However, prolonged action tetracycline hydrochloride products (29,30) are reported to be equally effective when compared to the conventional tablet in the treatment of



acne vulgaris and gonorrhoea. One of the prolonged action product (29) was reported to be effective even when one half the daily dosage compared to the conventional tablet was administered hence reducing the possible incidence of undesirable side effects.

B. HYDRALAZINE HYDROCHLORIDE

Procedure :

Four mongrel dogs of either sex, each weighing 8-12 kg, were fasted for 24 hrs prior to the administration of the samples. The study was carried out in crossover design. The dogs were weighed and anaesthetised with pentobarbitone sodium (31) (30 mg/kg i.p. and maintenance dose of 5 mg/kg i.v. was administered, when necessary). A gap was placed between the teeth, and the capsule/tablet containing drug was placed back in the throat by means of a forcep and washed with 100 ml of water. Blood sample (about 15 ml) was withdrawn from femoral vein, which was earlier canulated. Blood samples were collected at 0.5, 1, 3, 5, 6, 9, 12 hrs after administration of each sample. Sterile normal saline solution was infused prior to and after blood sampling. The blood samples were centrifuged immediately, the plasma was acidified and stored frozen until analysed employing the method described in Chapter II (page 49).

Observations are recorded in Tables 4-4 and 4-5 and shown graphically in Figure 4-3.

Statistical Analysis :

Average values of the plasma hydralazine levels with standard errors were calculated for each product, at every sampling time. The average plasma levels after administration of conventional and controlled release products were compared by paired t-test at every sampling time points. A similar analysis was performed on the area under the plasma concentration curves.

RESULTS AND DISCUSSION

Plasma Levels :

The mean plasma concentrations at every sampling time for each of the products, together with their standard errors, the areas under the curves and other pharmacokinetic parameters are given in Tables 4-4 and 4-5.

Analysis of these data at every sampling time points by paired t-test revealed the following significant differences ($P < 0.05$) :

1) The mean peak hydralazine plasma level was achieved more slowly and was lower when 50 mg of drug was administered in the controlled release product than in a 50 mg conventional capsule.

TABLE 4-4 : PLASMA LEVELS OF HYDRALAZINE AFTER ORAL ADMINISTRATION OF CONVENTIONAL AND CONTROLLED RELEASE PRODUCTS AND THEIR COMPARISON

Time Hrs	Mean Plasma levels ($\mu g\ ml^{-1}$)								Mean plasma levels compared by paired t-test (Level of significance)			
	a		b		c		d					
	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.	Mean	±S.E.	a vs b	a vs c	b vs d	c vs d
0.5	1.39	0.027	0.30	0.014	0.30	0.013	0.74	0.017	S	S	S	S
1.0	1.23	0.023	0.66	0.019	0.66	0.011	0.68	0.015	S	S	NS	NS
3.0	0.63	0.017	0.65	0.016	0.61	0.009	0.41	0.023	NS	NS	S	S
5.0	0.43	0.015	0.52	0.042	0.65	0.009	0.22	0.020	S	S	S	S
6.0	0.36	0.022	0.59	0.019	0.61	0.019	0.11	0.011	S	S	S	S
9.0	0.03	0.035	0.59	0.017	0.60	0.015	-	-	S	S	-	-
12.0	-		0.12	0.016	0.06	0.012	-		S	S	-	-

Key
a 50 mg conventional capsule
b 50 mg controlled release capsule
c 50 mg controlled release tablet
d 25 mg conventional capsule

S.E. = Standard Error
S - Significant at the 0.05 level
NS - Not Significant

FIG.·4-3: PLASMA LEVELS OF HYDRALAZINE AFTER ORAL ADMINISTRATION OF CONVENTIONAL AND CONTROLLED RELEASE PRODUCT.

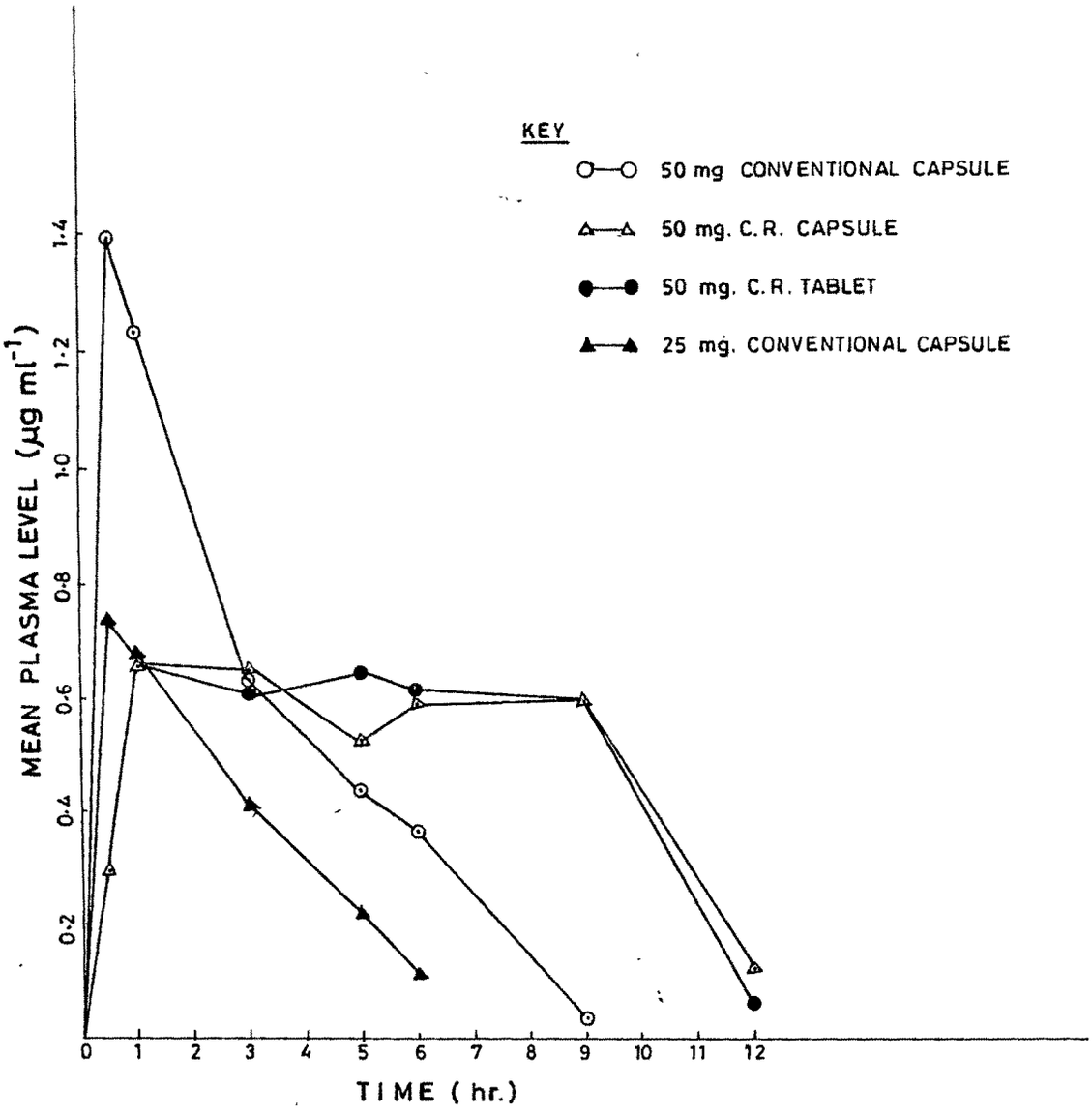


TABLE 4-5 : PHARMACOKINETIC PARAMETERS AFTER ORAL ADMINISTRATION OF CONVENTIONAL AND CONTROLLED RELEASE PRODUCTS OF HYDRALAZINE HYDROCHLORIDE

Product / Pharmacokinetic parameters	Mean[a]	± S.E.	Mean[b]	± S.E.	Mean[c]	± S.E.	Mean[d]	± S.E.
T_{max} (Hrs)	0.50	0.060	1.00	0.100	1.00	0.150	0.50	0.060
C_{max} ($\mu g\ ml^{-1}$)	1.39	0.029	0.66	0.016	0.66	0.011	0.74	0.016
AUC_{0-12} ($\mu g\ hr\ ml^{-1}$)	4.88	0.154	6.30	0.238	6.47	0.155	2.43	0.108
$\bar{C}$ ($\mu g\ ml^{-1}$)	0.41	0.013	0.53	0.020	0.54	0.013	0.40	0.018

Key
a 50 mg conventional capsule
b 50 mg controlled release capsule
c 50 mg controlled release tablet
d 25 mg conventional capsule

S.E. = Standard Error

T_{max} - Time required to achieve peak plasma concentration.

C_{max} - Peak plasma concentration.

AUC_{0-12} - Area under the plasma level curve 0 - 12 hours

$\bar{C}$ - AUC_{0-12} /dosing interval

2) The mean hydralazine plasma levels, attained between 5-12 hrs with controlled release products were higher than those achieved with 50 mg conventional capsule.

3) The mean hydralazine plasma levels between 3-6 hrs were higher with the controlled release products compared to plasma levels with the 25 mg conventional capsule.

4) Area under the hydralazine plasma level curve (AUC_{0-12}) after administration of each product was calculated using trapezoidal rule (26). Controlled release products had higher areas under plasma level curves compared to that of the conventional product.

5) Average plasma concentration for each product was calculated by dividing value of AUC_{0-12} for each product with dosing interval (32). Sustained release products have higher average hydralazine plasma concentration compared to conventional product.

The controlled release products gave lower and delayed peak drug concentrations, thus altering the plasma profile of hydralazine in a mode consistent with the sustained release characteristics claimed for these products. Higher and consistent plasma hydralazine concentrations are maintained between 5-12 hrs with controlled release products when compared with the conventional 50 mg capsule. Furthermore, average plasma concentration ($\bar{C}$) was found

significantly higher for controlled release products. This further proves consistent and higher hydralazine plasma concentration after administration of controlled release products.

The areas under the curves are significantly higher for controlled release products in comparison with those achieved by conventional hydralazine hydrochloride in single dose. This may be due to the rapid changes in plasma concentrations of the drug following the intake of the conventional capsule.

From the studies, the new controlled release products appear promising with regard to making medication simpler for hypertensive patients. However, as the suggestions have been raised that even conventional hydralazine hydrochloride tablets may be prescribed only twice daily (33), the role for controlled release hydralazine products in treatment of hypertension can only be settled from further clinical trials, with particular emphasis on the incidence of side effects. We would expect the slow release products to cause less side effects, but this could not be evaluated in the present study.

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