CHAPTER 5

ENHANCEMENT OF BIOAVAILABILITY OF SOME ANTIBIOTICS

5.1. Introduction

There is a great importance in the therapeutic regimen for the improvement of bioavailability of a large number of drugs which are (a) poorly biologically available (b) given for long periods, and (c) toxic and expensive. Maximizing oral bioavailability is therapeutically important as the extent of bioavailability directly influences plasma concentration and consequently therapeutic efficacy and dose related toxic effects resulting from oral administration. Any significant improvement in bioavailability will result in lowering the dose and the frequency of doses of that particular drug. Besides, inter-subject variability is inversely correlated with the extent of bioavailability.

Several approaches have been adopted in the past to maximize oral bioavailability. However, based on the clues from Ayurvedic literature, a new approach of increasing the bioavailability of drugs has been conceptualized [1]. There is a growing awareness that herbal medicines and other phytochemicals could severely affect the disposition of certain drugs and pharmaceuticals. Well-known examples are concurrent administration of St. John's wort rendering antiviral treatment less efficient and grapefruit juice increases the bioavailability of number of drugs [2]. Recent studies have shown that the presence of flavonoids, furanocoumarins and other phytoconstituents present in the grapefruit are found to be involved in the enhancement of therapeutic efficacy of number of drugs [3, 4]. It was determined that in combination with piperine the dose of rifampicin can be reduced by about 50% while retaining the therapeutic efficacy at par with the standard dose [5]. Based on these findings other reputed plants were evaluated for enhancing bioavailability of various drugs.

Amoxycillin and cefixime are β - lactam antibiotics exhibiting a marked bactericidal effect against different Gram-positive and Gram-negative bacteria. They are useful in the treatment of respiratory tract, urinary tract and soft tissue infection [6]. Rifampicin, the semisynthetic hydrazine derivatives of rifamycin B, is one of the most potent and powerful mycobactericidal drugs and used mainly for the treatment of tuberculosis and leprosy [7]. It is also indicated for the prophylactic treatment of *H.influenza* (type B)

causing meningitis. It is potent inducers of hepatic microsomal enzyme and its administration results in a decrease of its own half-life and number of other drugs [6].

All the three antibiotics are absorbed rapidly after oral administration, but, when taken with food, the rate and sometimes extent of absorption are decreased. Besides the systemic metabolism and poor patient compliance the insufficient rate and extent of absorption affected the success of their antibacterial therapies. After considering all these factors it was felt necessary for the structural and effective change in the regimen of these drug therapies. The present study reports the effect of methanol extract of *Colebrookea oppositifolia* leaf, *Heracleum nepalense* root and their isolated compounds on the bioavailability and pharmacokinetic of amoxycillin, cefixime and rifampicin in rabbits.

5.2. Materials and Methods

5.2.1. Plant materials

Methanol extracts of *Colebrookea oppositifolia* leaf and *Heracleum nepalense* root as well as their isolated compounds (described in **Chapter 3**) were used as test drug in these experiments.

5.2.2. Test compound formulations

Oral suspensions of the extract and isolated compounds were prepared by suspending them separately in 1% solution of sodium carboxy methylcellulose to prepare suitable dosage forms.

5.2.3. Drugs and chemicals

Amoxycillin was purchased from Libra Drugs (India) Ltd, Pune. Cefixime and rifampicin were kindly gifted by Blue Cross (India) Ltd, Mumbai and Lupin Laboratories Ltd, Pimpiri, Pune respectively. HPLC grade acetonitrile, methanol, water were purchased from S.D Fine Chemicals Ltd, Mumbai. Potassium dihydrogen phosphate, Potassium hydroxide and Zinc sulphate were obtained from Qualigens Fine Chemicals, Mumbai.

5.2.4. Animals

Albino rabbits weighing between 1.5 – 2 kg of either sex were used. They were housed under standard conditions of temperature (23 ±10°C) and relative humidity (55±10%); 12hr/12hr light/dark cycle and fed with standard pellet diet and water *ad libitum*. Rabbits were fasted for at least 24 hr prior to the experiments.

5.2.5. HPLC method of analysis of antibiotics

5.2.5.1. Chromatographic condition

An isocratic HPLC system (Shimadzu) consisting of LC – 20 at liquid pump, SPD – 20 AL UV – Visible detector and spinchrome software was used.

- **Column:** An ODS C-18 RP column (4.6 mml. D × 250 mm) with Hamilton 702 NR (25 µl) injecting syringe.
- **Mobile Phase:** Acetonitrile: Phosphate buffer (0.01M), The pH was adjusted to 5.0 with potassium hydroxide solution (45% w/v).
- **Column Temperature:** Ambient
- **Flow rate:** 1.0 ml/min
- **Injection volume:** 20 µl
- **Detector:** UV
- **Wave length:** 230 nm

5.2.5.2. Validation of assay method

The system suitability was evaluated by the intraday and interday precision and accuracy of triplicates. The accuracy of this method was further assessed with recovery study by spiking the antibiotics separately into blank plasma and phosphate buffer (pH 6) to afford

5.0, 10.0 and 20 μg/ml in triplicates, respectively, and the concentration obtained in blank plasma to the corresponding ones were compared. The LOQ (Limit of quantification) represents the lowest concentration of analysis in a sample that can be determined with acceptable precision and accuracy.

To verify the suitability of method the following analytical variables were analysed:

1. **Precision:** the precision of the method was assessed by repeated analysis of plasma containing known concentrations of all antibiotics separately.

2. **Recovery:** The absolute recovery from plasma was measured in the following way: The drug was added to drug free plasma to achieve the midpoint concentrations and were analysed carefully. Measured aliquot of the acetonitrile layer injected and the peak areas were measured. Absolute recovery was calculated by comparing these peak areas with the peak area obtained by the direct injection of the pure drug standard.

3. **Linearity and Sensitivity:** Concentration and peak area of standard antibiotics in plasma correlated linearly with each other.

5.2.5.3. Quantitative analysis

External standard calibration method was used for quantitative analysis of antibiotics. The external standard was the same substance as that being analysed in the plasma sample. In this method, by injecting standard solution of antibiotic in different concentrations, peak response was plotted versus concentration. Unknown samples were analysed in similar manner and their concentrations were determined from calibration curve. The calibration curve has well covered the range of unknown sample.

$$R_f = \frac{\text{Standard peak area}}{\text{Concentration of the sample}}$$

$$\text{Unknown concentration} = \frac{\text{Peak response of the sample}}{R_f}$$

5.2.6. Preparation of standard curve of amoxycillin, cefixime and rifampicin.

Thawed and drug free plasma from rabbit was pipetted into a disposable test tube and spiked with 50 µl of standard solution of amoxycillin to make the concentration of the drug up to 10, 20, 40, 60, 80, 100, 120 µg/ml of solution with distilled water. The solutions were vortex mixed for 30 sec. After the addition of 300 µl HPLC grade methanol and 200 µl zinc sulphate (0.7M), the tubes were vortex mixed for 30 sec and then centrifuged at 3000 rpm for 5 mins. A 20 µl aliquot of the supernatant was injected into HPLC system and eluted with mobile phase at the rate of 1.0 ml/min at ambient temperature. The column output is monitored at 230 nm using UV detector. The standard curve with peak area on Y-axis and concentration on X-axis was prepared. Similar procedure was followed for preparation of standard curve of cefixime and rifampicin.

5.2.7. Effect of *C.oppositifolia* leaf extract and compound I on bioavailability and pharmacokinetics of antibiotics.

5.2.7.1. Administration schedule of test drugs.

Rabbits were divided into seven groups, each containing six animals. Group I served as control and received only amoxycillin at a dose of 100 mg/kg, p.o. Group II was coadministered 450 mg/kg, p.o. dose of methanol extract of *C.oppositifolia*, and group III & IV were coadministered 25 mg/kg and 50 mg/kg, p.o. dose of compound I respectively with amoxycillin at a dose of 100 mg/kg. Group V – VII were given 450 mg/kg, p.o. dose of methanol extract of *C.oppositifolia*, 25 mg/kg and 50 mg/kg, p.o. dose of compound I respectively and 30 mins later amoxycillin trihydrate was administered at a dose of 100 mg/kg, p.o. Similar type of treatment schedule was followed for cefixime and rifampicin.

The dose of amoxycillin, cefixime and rifampicin was chosen as 100 mg/kg to keep plasma concentrations above the lower limit of detection. As the minimum lethal dose of methanol extract of *C.oppositifolia* was reported to be 4.5 g/kg body weight (described in **Chapter 4**), one tenth of the MLD was selected for evaluation of the activity [8]. Compound I was tested at different doses (25 and 50 mg/kg) for evaluation of the activity.

5.2.7.2. Preparation of plasma samples and determination of plasma levels of antibiotics

Each rabbit was anaesthetized with ether. The right ear marginal vein was cannulated with polyethylene tubing for blood sampling. Blood samples (2 ml) were withdrawn at 0, 0.5, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0 hrs after administration of the drugs and centrifuged at 5000 rpm for 5 min to obtain the plasma samples (1 ml). The samples were labeled properly and stored at – 20°C up to the HPLC analysis.

Thawed plasma samples (900 µl) were mixed with 300 µl HPLC grade methanol and 200 µl zinc sulphate solution (0.7 M). The mixtures were centrifuged at 3000 rpm for 5 min and the supernatants were (20 µl) assayed in the same manner as described in the preparation of standard curve of antibiotics in plasma.

5.2.7.3. Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (t_{max}) were determined by a visual inspection of the experimental data. The area under the plasma concentration-time curve (AUC) was calculated by trapezoidal rule method. The elimination rate constant (k_{el}) was calculated by regression analysis from the slope of the line, and the half -life ($t_{1/2}$) of the antibiotics was obtained by applying the equation $0.693/k_{el}$. The absorption rate constant (k_a) was calculated by the residual method. The relative bioavailability of the antibiotics (RB%) after oral administration was calculated as follows:

$$\text{Relative bioavailability (RB\%)} = \frac{AUC_{\text{coadministered group}}}{AUC_{\text{control group.}}} \times 100$$

5.2.8. Effect of *H.nepalense* root extract and compound II on bioavailability and pharmacokinetics of antibiotics.

The effect of methanol extract of *H.nepalense* root and its isolated compound II on bioavailability and pharmacokinetics of the tested antibiotics was determined as per the methods described above in **5.2.7** for *C.oppositifolia* leaf.

5.3. Results

5.3.1. Effects of *C.oppositifolia* leaf extract and compound I on bioavailability and pharmacokinetics of antibiotics.

The HPLC method of analysis for amoxycillin, cefixime and rifampicin in rabbit plasma was developed and validated. The limit of quantification was 0.2 μg/ml with recovery >90% for all the three antibiotics. The coefficient of variation for within a day precision ranged from 0.6 to 9.6%. The percent relative standard deviation (% RSD) was calculated and found to be 0.05% (Table 5.1 to 5.3). The coefficient of correlation of the assay was found to be 0.999 in amoxycillin, cefixime and rifampicin. Chromatographic elution was undertaken for 30 mins and the average retention time for amoxycillin, cefixime and rifampicin were about 2.6, 3.9 and 6.9 min respectively (Figure 5.1 to Figure 5.3). The plasma concentrations of all antibiotics after administration of test compounds were calculated from the standard calibration curve as presented in Table 5.4 to Table 5.6 and Figure 5.4 to Figure 5.5.

The plasma concentration-time profile after oral administration of amoxycillin with or without *C.oppositifolia* and compound I is presented in Table 5.7. The data was fitted to a one compartment open model, which followed first order kinetics. Comparative pharmacokinetic parameters calculated from these data are summarized in Table 5.8. In all types of the administration absorption process was completed within median t_{max} 1.5 hr. When amoxycillin (100 mg/kg) were co-administered or given 30 min after the administration of methanol leaf extract peak plasma concentration C_{max} was found to be 6.14 ± 0.39 μg/ml and 6.72 ± 1.04 μg/ml respectively as compared to 5.28 ± 0.32 μg/ml for the control group. But the preadministration of methanol leaf extract shifted the t_{max} 0.5 hr earlier than the control group (1.0 ± 0.41 hr versus 1.5 ± 0.62 hr). The relative bioavailability of amoxycillin preadministered with methanol leaf extract (211%) was higher than the co-administration of the same dose of methanol leaf extract (163%). Comparison of other pharmacokinetic parameters of both the groups with control showed significant difference in plasma half-life ($t_{1/2}$) and elimination rate constant (k_{el}). The absorption rate constants (k_a) of both co-administered and preadministered with methanol

extract groups were increased compared with the control but not statistically significant. When compound I was co-administered at 25 and 50 mg/kg dose with amoxycillin at 100 mg/kg, C_{max} was determined to be 6.52 ± 0.52 μg/ml and 6.71 ± 0.12 μg/ml respectively. In addition, the t_{max} was attained 1.0 hr sooner than the t_{max} for the control group. The C_{max} values of amoxycillin was found to be increased in a dose dependent manner when co-administered or preadministered with compound I at 25 and 50 mg/kg body weight. The absorption rate constants (k_a) of amoxycillin were increased but not statistically significant. Comparison of AUC showed that higher plasma levels of amoxycillin were achieved in the group administered with compound I and amoxycillin 30 min after. The relative bioavailability (RB %) of amoxycillin in preadministered group with compound I at a dose of 50 mg/kg is higher than the co-administered group of the same compound at the same dose. Comparison of other pharmacokinetic parameters with co-administered or preadministered group showed significant increase in elimination half-life ($t_{1/2}$) and elimination rate constant (k_{el}) than the control group.

The result outlined in Table 5.9 showing the mean plasma concentration versus time profile of cefixime administration alone and in combination with methanol extract of *C.oppositifolia* and compound I. The bioavailability and the pharmacokinetic parameters of cefixime after co-administration and preadministered with methanol leaf extract and compound I are shown in Table 5.10. Following co-administration and preadministration of methanol extract of *C.oppositifolia* resulted the peak plasma concentration C_{max} of 4.03 ± 0.42 μg/ml and 4.12 ± 0.22 μg/ml respectively as compared to the control group where it is found to be 4.01 ± 0.36. The time to reach maximum concentration (t_{max}) failed to show any significant differences as compared with the control. Comparison of other pharmacokinetic parameters also failed to show any significant differences in the relative bioavailability (RB%), absorption rate constant (k_a) and mean elimination half- life ($t_{1/2}$). There is no significant difference between the respective AUC values of the groups administered with the test compared with the control group. However the preadministration of compound I 30 mins before the administration of cefixime (100 mg/kg) increased the C_{max} (4.93 ± 0.64 μg/ml at 25 mg/kg and 5.21 ± 0.24 μg/ml at 50 mg/kg); $t_{1/2}$ (3.64 ± 0.61 hr at 25 mg/kg and 4.0 ± 0.32 hr at 50 mg/kg) and AUC (18.05 ±

1.62 μg.hr/ml at 25 mg/kg and 21.06 ± 2.56 μg.hr/ml at 50 mg/kg) as compared to the control group (C_{max} 4.01± 0.36 μg/ml, $t_{1/2}$ 3.01 ± 0.21 hr, AUC 15.17 ± 3.58 μg.hr/ml). The relative bioavailability (RB%) of the cefixime with the preadministered compound I at 50 mg/kg was higher (139%) than the co-administered group at the same dose of compound I (106%).

The mean plasma concentration versus time profile of rifampicin administration with or without methanol extract of *C.oppositifolia* (350 mg/kg), compound I (25mg/kg) is shown in Table 5.11. The data fitted to a one compartment open model, which followed the first order kinetic, and other pharmacokinetic patterns derived from these data are summarized in Table 5.12. In all type of administration the absorption process was completed with t_{max} of 2.5 ± 0.78 hr, 2.0 ± 0.34 hr and 1.5 ± 0.36 hr for rifampicin (100 mg/kg), rifampicin (100 mg/kg) coadministered with methanol extract of *C.oppositifolia* (450 mg/kg) and rifampicin (100 mg/kg) after 30 min of administration of methanol extract of *C.oppositifolia* (450 mg/kg) respectively. The methanol extract of *C.oppositifolia* preadministration induced a significant shift in C_{max} of rifampicin, which was statistically significant ($P<0.05$). The distribution phase was fairly short in all the groups and a fall of concentration of the antibiotic being evident within 2.5 hr of drug administration. Comparison of other pharmacokinetic parameters of the coadministered & preadministered groups with the control showed significant increases in the C_{max} and the mean elimination half-life ($t_{1/2}$). Comparison of AUC showed that higher plasma level of rifampicin was achieved in the group administered with methanol extract of *C.oppositifolia* and postadministration of rifampicin (30 min). The relative bioavailability (RB %) of rifampicin was increased in the groups co-administered and preadministration with methanol extract compared with the control. The absorption rate constants (k_a) of rifampicin were also increased but not statistically significant in both the groups. Following co-administration and preadministration of compound I at 25 and 50 mg/kg the C_{max} was further increased significantly to 8.14 ± 0.84 μg/ml and 8.67 ± 0.52 μg/ml with t_{max} of 2.0 ± 0.32 hr and 1.5 ± 0.31 hr respectively. The half-life ($t_{1/2}$) was prolonged significantly in the groups where compound I was administered 30 min before the administration of rifampicin. All other pharmacokinetic parameters including AUC,

which was increased, and the k_{el}, which was decreased significantly when compound I was preadministered and co-administered with rifampicin compared to the control group. The absorption rate constants (k_a) of both co-administered and preadministered group with compound I were increased compared to the control but not statistically significant.

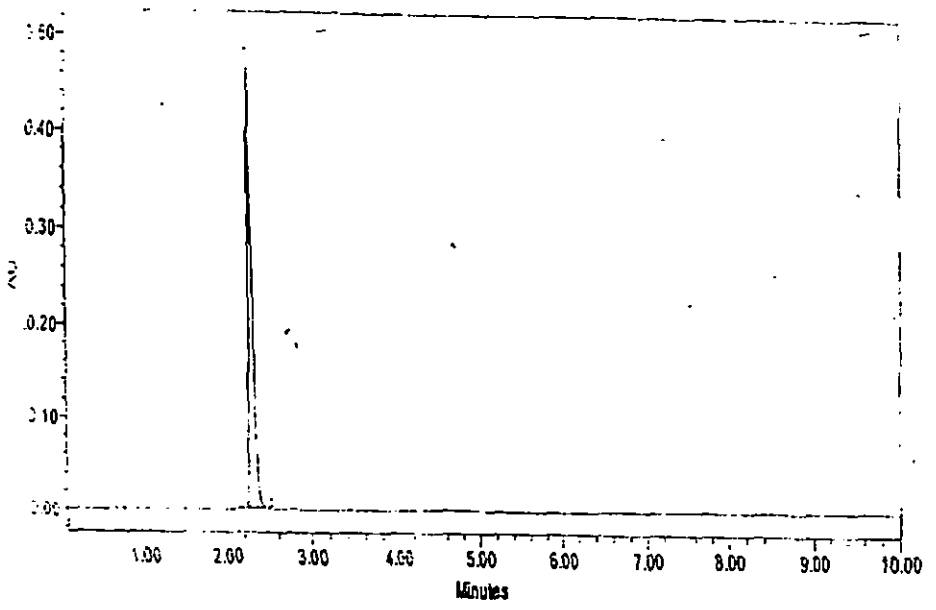

Figure 5.1. Typical HPLC Chromatogram of amoxycillin.

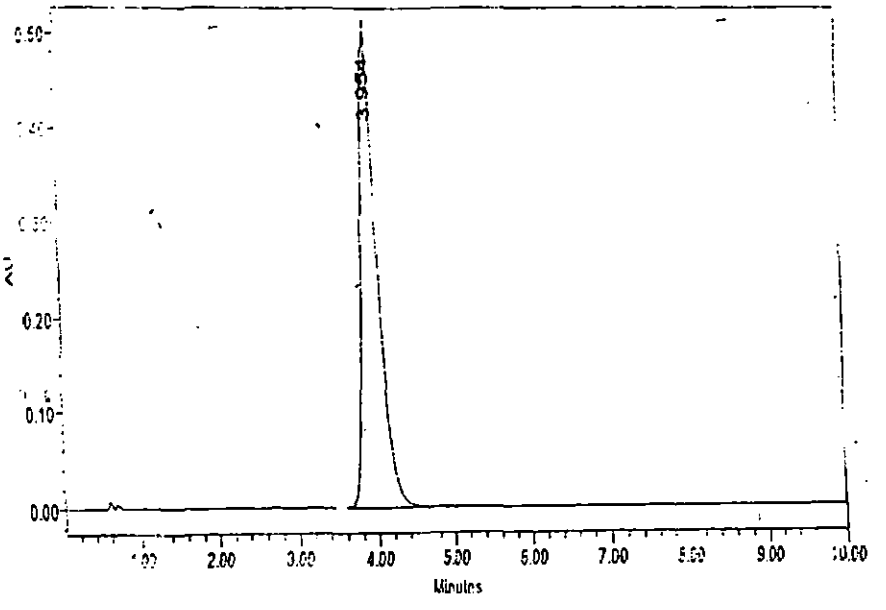

Figure 5.2. Typical HPLC Chromatogram of cefixime.

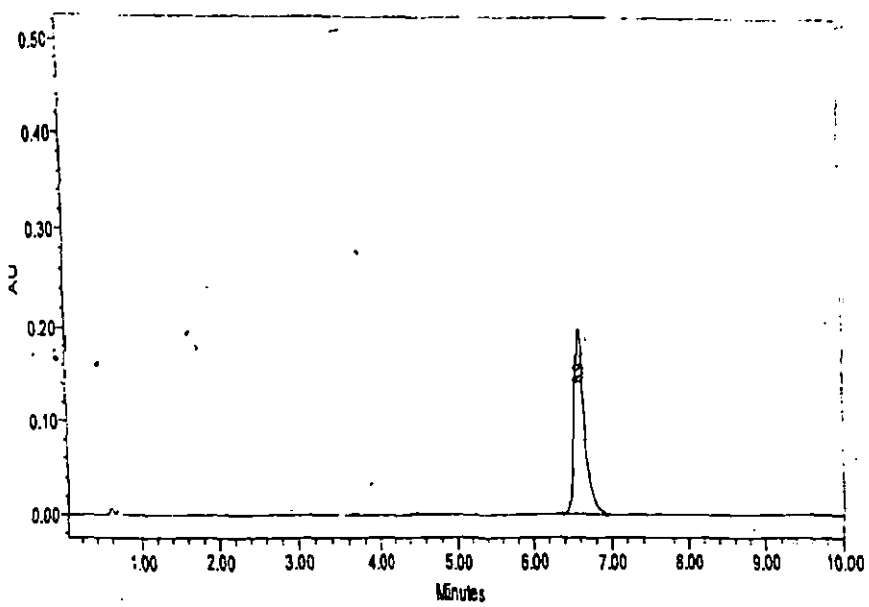

Figure 5.3. Typical HPLC Chromatogram of rifampicin.

Table 5.1 Percent relative standard deviation of amoxycillin.

Injection number	Retention time (min)	Peak area observed
1	2.60	3680192
2	2.61	3679562
3	2.60	3680242
4	2.59	3682469
5	2.60	3678292

Concentration used to calculate % RSD is 10 µg/ml

$$\% \text{ RSD} = \frac{\text{Average peak area} - \text{Minimum peak area}}{\text{Average peak area}}$$

Average peak area = 3680151, Minimum peak area = 36782922

% RSD = 0.05

Table 5.2 Percent relative standard deviation of cefixime.

Injection number	Retention time (min)	Peak area observed
1	3.41	3597423
2	3.40	3596727
3	3.41	3597872
4	3.42	3595512
5	3.40	3599621

Concentration used to calculate % RSD is 10 µg/ml

$$\% \text{ RSD} = \frac{\text{Average peak area} - \text{Minimum peak area}}{\text{Average peak area}}$$

Average peak area = 3597431, Minimum peak area = 3595512

% RSD = 0.05

Table 5.3 Percent relative standard deviation of rifampicin.

Injection number	Retention time (min)	Peak area observed
1	5.92	3694178
2	5.92	3696232
3	5.90	3694583
4	5.91	3694247
5	5.92	3692352

Concentration used to calculate % RSD is 10 µg/ml

$$\% \text{ RSD} = \frac{\text{Average peak area} - \text{Minimum peak area}}{\text{Average peak area}}$$

Average peak area = 3694318, Minimum peak area = 3692352

% RSD = 0.05

Table 5.4 Data for standard graph of amoxycillin in plasma.

Sl. No.	Concentration (µg/ml)	Mean peak area
1	1	365154.37
2	2	742367.24
3	3	1181562.72
4	4	1486482.62
5	5	1902923.54
6	10	3680195.30
7	15	6169852.13
8	20	7323146.77

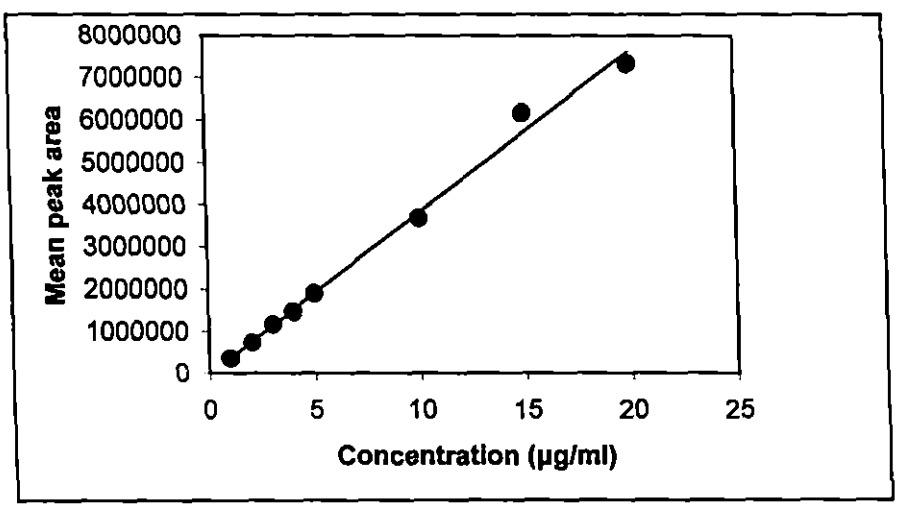

Figure 5.4. Standard curve of amoxycillin in plasma.

Table 5.5 Data for standard graph of cefixime in plasma.

Sl. No.	Concentration (µg/ml)	Mean peak area
1	1	352642.14
2	2	714624.55
3	3	1162436.11
4	4	1483727.57
5	5	1832420.66
6	10	3598969.18
7	15	6437894.30
8	20	7290055.15

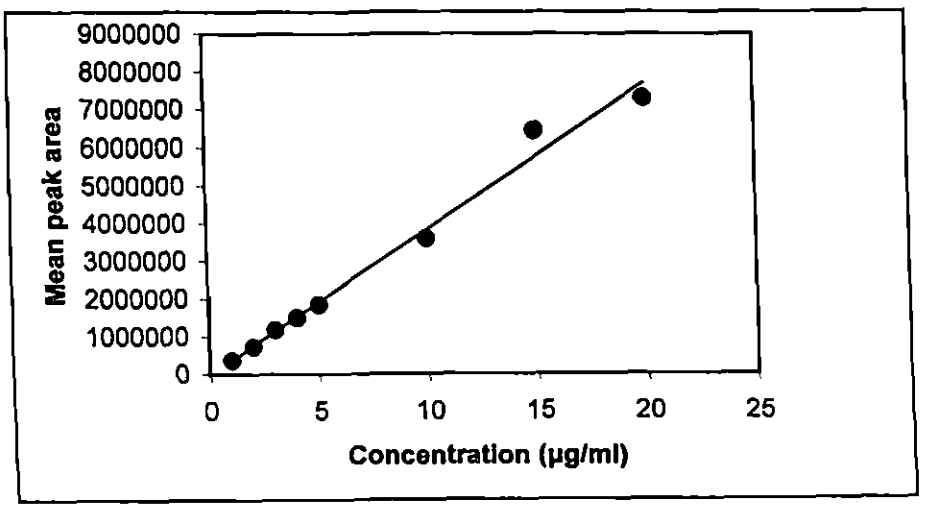

Figure 5.5. Standard curve of cefixime in plasma.

Table 5.6 Data for standard graph of rifampicin in plasma.

Sl. No.	Concentration (µg/ml)	Mean peak area
1	1	388819.52
2	2	777254.24
3	3	1106453.62
4	4	1481278.12
5	5	1826435.62
6	10	3693142.32
7	15	6242572.39
8	20	7369821.66

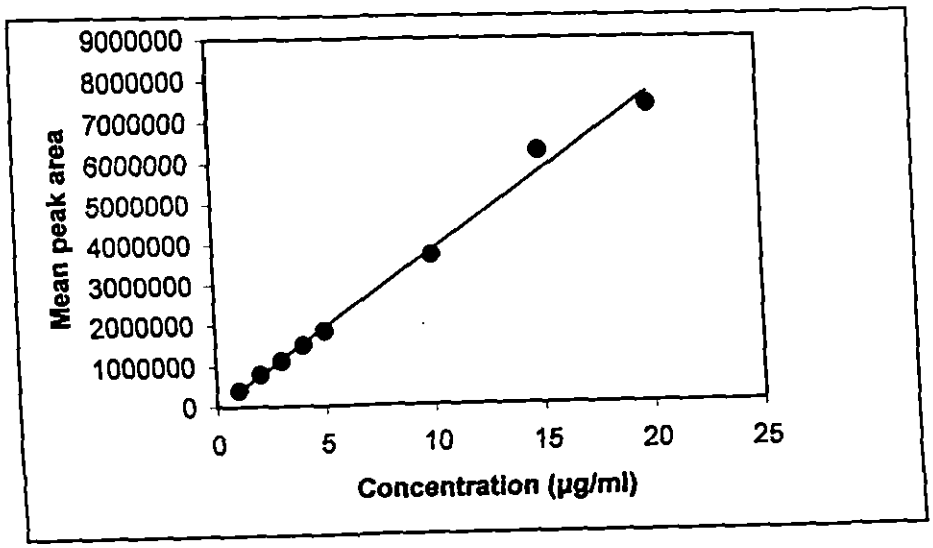

Figure 5.6. Standard curve of rifampicin in plasma.

Table 5.7 Mean plasma concentration of amoxycillin after oral administration of amoxycillin (100 mg/kg), co-administration with methanol extract of *C.oppositifolia* (450 mg/kg) and compound I (25, 50 mg/kg) and preadministered with *C.oppositifolia* (450 mg/kg) and compound I (25, 50 mg/kg) in rabbit.

Sl. No	Time of collection (hr)	(Group I) Amoxycillin (100 mg/kg)	(Group II) methanol extract (450 mg/kg) + Amoxycillin (100 mg/kg)	(Group III) Compound I (25 mg/kg) + Amoxycillin (100 mg/kg)	(Group IV) Compound I (50 mg/kg) + Amoxycillin (100 mg/kg)	(Group V) methanol extract (450 mg/kg) + Amoxycillin (100 mg/kg) 30 min later	(Group VI) Compound I (25 mg/kg) + Amoxycillin (100 mg/kg) 30 min later	(Group VI) Compound I (50 mg/kg) + Amoxycillin (100 mg/kg) 30 min later
1	0.5	1.67 ± 0.191	2.12 ± 0.231	2.86 ± 0.112	3.06 ± 0.352	3.84 ± 0.392	3.09 ± 0.154	3.85 ± 0.212
2	1.0	3.12 ± 0.393	3.86 ± 0.352	4.62 ±0. 321	5.12 ± 0.252	6.72 ± 1.041	7.07 ± 0.742	7.51 ± 0.332
3	1.5	5.26 ± 0.323	6.14 ± 0.391	6.52 ± 0.522	6.71 ± 0.121	6.08 ± 0.726	6.45 ± 0.332	6.87 ± 0.543
4	2.0	4.23 ± 0.546	5.84 ± 0.655	5.79 ± 0.423	6.03 ± 0.211	5.83 ± 0.521	5.94 ± 0.141	6.06 ± 0.412
5	2.5	3.56 ± 0.522	4.62 ± 0.312	4.82 ± 0.152	5.01 ± 0.432	4.92 ± 0.132	5.04 ± 0.112	5.28 ± 0.645
6	3.0	2.34 ± 0.761	3.17 ± 0.231	3.82 ± 0.111	4.67 ± 0.311	3.86 ± 0.432	4.69 ± 0.462	4.16 ± 0.322
7	4.0	1.33 ± 0.234	2.22 ± 0.145	2.74 ± 0.243	3.68 ± 0.512	3.12 ± 0.321	3.71 ± 0.562	3.67 ± 0.515
8	5.0	1.12 ± 0.645	1.84 ± 0.532	2.12 ± 0.521	2.74 ± 0.234	2.46 ± 0.645	2.75 ± 0.732	2.92 ± 0.843
9	6.0	0.92 ± 0.074	1.12 ± 0.191	1.34 ± 0.512	1.94 ± 0.512	1.92 ± 0.685	1.93 ± 0.113	2.03 ± 0.752
10	8.0	0.46 ± 0.012	0.96 ± 0.321	0.99 ± 0.015	1.02 ± 0.112	1.24 ± 0.213	1.03 ± 0.115	1.16 ± 0.119
11	10.0	0.29 ± 0.065	0.82 ± 0.031	0.64 ± 0.034	0.78 ± 0.055	0.98 ± 0.012	0.72 ± 0.032	0.89 ± 0.016
12	12.0	0.11 ± 0.087	0.49 ± 0.012	0.52 ± 0.021	0.54 ± 0.012	0.64 ± 0.032	0.56 ± 0.011	0.74 ± 0.042
13	24.0	0.03 ± 0.009	0.22 ± 0.011	0.21 ± 0.012	0.22 ± 0.021	0.44 ± 0.011	0.22 ± 0.017	0.24 ± 0.043

Values are expressed in µg/ml, and mean ± SEM; n=6 in each group. $P<0.05$ in comparison with control

Table 5.8 Comparison of pharmacokinetic parameters of amoxycillin alone and in combination with methanol extract of *C.oppositifolia* and compound I

Sl.No	Parameters	(Group I) Amoxycillin (100 mg/kg)	(Group II) methanol extract (450 mg/kg) + Amoxycillin (100 mg/kg)	(Group III) Compound I (25 mg/kg) + Amoxycillin (100 mg/kg)	(Group IV) Compound I (50 mg/kg) + Amoxycillin (100 mg/kg)	(Group V) methanol extract (450 mg/kg) + Amoxycillin (100 mg/kg) 30 min later	(Group VI) Compound I (25 mg/kg) + Amoxycillin (100 mg/kg) 30 min later	(Group VI) Compound I (50 mg/kg) + Amoxycillin (100 mg/kg) 30 min later
1	C_{max} (µg/ml)	5.28 ± 0.32	6.14 ± 0.39	6.52 ± 0.52	6.71 ± 0.12	6.72 ± 1.04	7.07 ± 0.74	7.51 ± 0.33
2	t_{max} (hr)	1.5 ± 0.61	1.5 ± 0.31	1.5 ± 0.42	1.5 ± 0.54	1.0 ± 0.41	1.0 ± 0.40	1.0 ± 0.36
3	AUC (µg.hr/ml)	17.32 ± 2.34	28.05 ± 1.81	30.1 ± 2.59	34.43 ± 3.36	36.59 ± 3.21	35.38 ± 2.46	38.20 ± 1.92
4	$t_{1/2}$ (hr)	1.54 ± 0.47	1.69 ± 0.43	1.72 ± 0.63	1.84 ± 0.67	1.83 ± 0.32	2.13 ± 0.39	2.34 ± 0.67
5	k_{el} (hr^{-1})	0.45 ± 0.023	0.41 ± 0.024	0.40 ± 0.036	0.37 ± 0.041	0.37 ± 0.032	0.32 ± 0.021	0.29 ± 0.051
6	RB (%)	100	163	173	199	211	204	221
7	k_a (hr^{-1})	2.71 ± 0.32	2.83 ± 0.41^{NS}	2.95 ± 1.11^{NS}	2.97 ± 0.83^{NS}	2.96 ± 1.12^{NS}	3.09 ± 1.10^{NS}	3.23 ± 0.43^{NS}

Values are in mean ± SEM; n=6 in each group; P<0.05 in comparison with control; C_{max}: peak concentration; t_{max}: time to reach peak concentration; AUC: area under plasma concentration time curve from 0 hrs to 24 hr; $t_{1/2}$: plasma half life; k_{el}: elimination rate constant; RB (%): relative bioavailability; ka: absorption rate constant; NS: not significant.

Table 5.9 Mean plasma concentration of cefixime after oral administration of cefixime (100 mg/kg), co-administration with methanol extract of *C.oppositifolia* (450 mg/kg) and compound I (25, 50 mg/kg) and preadministered with *C.oppositifolia* (450 mg/kg) and compound I (25, 50 mg/kg) in rabbit.

Sl. No	Time of collection (hr)	(Group I) Cefixime (100 mg/kg)	(Group II) methanol extract (450 mg/kg) + cefixime (100 mg/kg)	(Group III) Compound I (25 mg/kg) + cefixime (100 mg/kg)	(Group IV) Compound I (50 mg/kg) + cefixime (100 mg/kg)	(Group V) methanol extract (450 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VI) Compound I (25 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VII) Compound I (50 mg/kg) + cefixime (100 mg/kg) 30 min later
1	0.5	1.76 ± 0.142	1.78 ± 0.232	1.56 ± 0.242	1.82 ± 0.374	1.84 ± 0.462	1.94 ± 0.413	3.32 ± 0.469
2	1.0	2.98 ± 0.132	2.97 ± 0.231	2.86 ±0. 232	2.99 ± 0.371	2.98 ± 0.241	3.23 ± 0.422	5.12 ± 0.243
3	1.5	4.01 ± 0.362	4.03 ± 0.426	3.97 ± 0.412	4.11 ± 0.251	4.12 ± 0.221	4.93 ± 0.645	4.92 ± 0.453
4	2.0	3.56 ± 0.546	3.69 ± 0.131	3.54 ± 0.321	3.63 ± 0.653	3.61 ± 0.582	3.98 ± 0.411	3.93 ± 0.142
5	2.5	2.84 ± 0.612	2.31 ± 0.321	3.04 ± 0.523	3.09 ± 0.184	3.01 ± 0.312	3.17 ± 0.237	3.19 ± 0.512
6	3.0	2.01 ± 0.341	1.94 ± 0.124	2.28 ± 0.234	2.77 ± 0.322	2.34 ± 0.322	2.46 ± 0.612	2.48 ± 0.242
7	4.0	1.92 ± 0.541	1.86 ± 0.245	1.94 ± 0.463	1.98 ± 0.157	1.96 ± 0.301	2.04 ± 0.643	2.02 ± 0.145
8	5.0	1.12 ± 0.523	1.02 ± 0.342	1.08 ± 0.341	1.06 ± 0.321	1.05 ± 0.146	1.29 ± 0.342	1.74 ± 0.413
9	6.0	0.84 ± 0.024	0.72 ± 0.431	0.84 ± 0.031	0.84 ± 0.101	0.81 ± 0.021	0.98 ± 0.043	1.03 ± 0.212
10	8.0	0.31 ± 0.032	0.31 ± 0.262	0.42 ± 0.046	0.56 ± 0.039	0.49 ± 0.014	0.67 ± 0.054	0.86 ± 0.019
11	10.0	0.19 ± 0.015	0.18 ± 0.042	0.23 ± 0.061	0.24 ± 0.051	0.21 ± 0.014	0.34 ± 0.036	0.39 ± 0.014
12	12.0	0.08 ± 0.037	0.09 ± 0.002	0.07 ± 0.007	0.09 ± 0.011	0.07 ± 0.005	0.11 ± 0.026	0.14 ± 0.010
13	24.0	0.01 ± 0.005	0.01 ± 0.005	0.03 ± 0.004	0.01 ± 0.002	0.02 ± 0.011	0.02 ± 0.005	0.03 ± 0.002

Values are expressed in μg/ml, and mean ± SEM; n=6 in each group. $P<0.05$ in comparison with control

Table 5.10 Comparison of pharmacokinetic parameters of cefixime alone and in combination with methanol extract of *C.oppositifolia* and compound I

Sl.No	Parameters	(Group I) Cefixime (100 mg/kg)	(Group II) methanol extract (450 mg/kg) + cefixime (100 mg/kg)	(Group III) Compound I (25 mg/kg) + cefixime (100 mg/kg)	(Group IV) Compound I (50 mg/kg) + cefixime (100 mg/kg)	(Group V) methanol extract (450 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VI) Compound I (25 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VII) Compound I (50 mg/kg) + cefixime (100 mg/kg) 30 min later
1	C_{max} (µg/ml)	4.01 ± 0.36	4.03 ± 0.42	3.97 ± 0.41	4.11 ± 0.25	4.12 ± 0.22	4.93 ± 0.64	5.12 ± 0.24
2	t_{max} (hr)	1.5 ± 0.42	1.5 ± 0.32	1.5 ± 0.24	1.5 ± 0.44	1.5 ± 0.31	1.5 ± 0.41	1.0 ± 0.21
3	AUC (µg.hr/ml)	15.17 ± 3.58	14.49 ± 2.65	15.41 ± 3.61	16.17 ± 3.34	15.44 ± 2.65	18.05 ± 1.62	21.06 ± 2.56
4	$t_{1/2}$ (hr)	3.01 ± 0.21	2.97 ± 0.35	3.01 ± 0.17	3.15 ± 0.52	3.01 ± 0.23^{NS}	3.64 ± 0.61	4.0 ± 0.32
5	k_{el} (hr^{-1})	0.23 ± 0.062	0.22 ± 0.024	0.23 ± 0.042	0.21 ± 0.032	0.23 ± 0.034	0.19 ± 0.016	0.17 ± 0.015
6	RB (%)	100	96	101	106	101	119	139
7	k_a (hr^{-1})	2.31 ± 0.16	2.29 ± 0.87^{NS}	2.31 ± 0.27	2.34 ± 0.83^{NS}	2.32 ± 0.42^{NS}	2.43 ± 1.10	2.86 ± 0.52

Values are in mean ± SEM; n=6 in each group; P<0.05 in comparison with control; C_{max}: peak concentration; t_{max}: time to reach peak concentration; AUC: area under plasma concentration time curve from 0 hrs to 24 hr; $t_{1/2}$: plasma half life; k_{el}: elimination rate constant; RB (%): relative bioavailability; ka: absorption rate constant; NS: not significant

Table 5.11 Mean plasma concentration of rifampicin after oral administration of rifampicin (100 mg/kg), co-administration with methanol extract of *C.oppositifolia* (450 mg/kg) and compound I (25, 50 mg/kg) and preadministered with *C.oppositifolia* (450 mg/kg) and compound I (25, 50 mg/kg) in rabbit.

Sl. No	Time of collection (hr)	(Group I) Rifampicin (100 mg/kg)	(Group II) methanol extract (450 mg/kg) + rifampicin (100 mg/kg)	(Group III) Compound I (25 mg/kg) + rifampicin (100 mg/kg)	(Group IV) Compound I (50 mg/kg) + rifampicin (100 mg/kg)	(Group V) methanol extract (450 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VI) Compound I (25 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VII) Compound I (50 mg/kg) + rifampicin (100 mg/kg) 30 min later
1	0.5	2.62 ± 0.143	3.14 ± 0.356	3.42 ± 0.126	4.17 ± 0.318	3.32 ± 0.311	3.89 ± 0.486	4.67 ± 0.238
2	1.0	3.52 ± 0.241	4.72 ± 0.278	4.91 ±0. 158	5.23 ± 0.247	4.82 ± 0.416	5.17 ± 0.422	7.18 ± 0.247
3	1.5	4.14 ± 0.221	7.06 ± 0.369	7.12 ± 0.242	7.37 ± 0.251	7.97 ± 0.672	7.99 ± 0.236	8.67 ± 0.524
4	2.0	5.82 ± 0.152	7.87 ± 0.681	7.89 ± 0.412	8.14 ± 0.846	7.36 ± 0.415	7.42 ± 0.162	7.98 ± 0.126
5	2.5	6.97 ± 1.321	6.98 ± 0.247	6.99 ± 0.177	7.88 ± 0.415	7.03 ± 0.423	7.07 ± 0.134	7.16 ± 0.443
6	3.0	6.12 ± 0.243	6.15 ± 0.244	6.18 ± 0.158	7.02 ± 0.151	6.29 ± 0.314	6.37 ± 0.434	6.67 ± 0.242
7	4.0	5.66 ± 0.748	5.72 ± 0.243	5.71 ± 0.434	6.16 ± 0.126	5.82 ± 0.231	5.94 ± 0.552	5.96 ± 0.145
8	5.0	4.72 ± 0.417	4.87 ± 0.381	4.88 ± 0.566	5.31 ± 0.458	4.99 ± 0.498	5.11 ± 0.342	5.16 ± 0.241
9	6.0	3.54 ± 0.726	3.96 ± 0.248	3.89 ± 0.124	4.23 ± 0.268	4.03 ± 0.523	4.14 ± 0.177	4.24 ± 0.284
10	8.0	2.32 ± 0.476	2.46 ± 0.252	2.52 ± 0.146	3.13 ± 0.142	3.02 ± 0.213	3.16 ± 0.162	3.52 ± 0.163
11	10.0	1.01 ± 0.242	1.06 ± 0.310	1.07 ± 0.328	1.37 ± 0.246	1.42 ± 0.172	1.51 ± 0.034	2.32 ± 0.136
12	12.0	0.62 ± 0.043	0.67 ± 0.014	0.68 ± 0.021	0.74 ± 0.012	0.94 ± 0.071	1.01 ± 0.013	1.21 ± 0.128
13	24.0	0.11 ± 0.027	0.14 ± 0.016	0.17 ± 0.013	0.23 ± 0.013	0.17 ± 0.026	0.19 ± 0.012	0.24 ± 0.021

Values are expressed in µg/ml, and mean ± SEM; n=6 in each group. $P<0.05$ in comparison with control

Table 5.12 Comparison of pharmacokinetic parameters of rifampicin alone and in combination with methanol extract of *C.oppositifolia* and compound I

Sl.No	Parameters	(Group I) Rifampicin (100 mg/kg)	(Group II) methanol extract (450 mg/kg) + rifampicin (100 mg/kg)	(Group III) Compound I (25 mg/kg) + rifampicin (100 mg/kg)	(Group IV) Compound I (50 mg/kg) + rifampicin (100 mg/kg)	(Group V) methanol extract (450 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VI) Compound I (25 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VII) Compound I (50 mg/kg) + rifampicin (10C mg/kg) 30 min later
1	C_{max} (µg/ml)	6.97 ± 1.32	7.87 ± 0.68	7.89 ± 0.41	8.14 ± 0.84	7.97 ± 0.67	7.99 ± 0.23	8.67 ± 0.52
2	t_{max} (hr)	2.5 ± 0.78	2.0 ± 0.34	2.0 ± 0.22	2.0 ± 0.32	1.5 ± 0.36	1.5 ± 0.24	1.5 ± 0.31
3	AUC (µg.hr/ml)	43.37 ± 2.43	48.56 ± 3.56	49.06 ± 2.59	54.94 ± 2.36	53.20 ± 1.42	55.33 ± 2.17	61.22 ± 3.82
4	$t_{1/2}$ (hr)	3.01 ± 0.45	3.31 ± 0.43	3.32 ± 0.63^{NS}	3.62 ± 0.64^{NS}	3.46 ± 0.47	3.74 ± 0.72	3.93 ± 0.74
5	k_{el} (hr^{-1})	0.23 ± 0.016	0.21 ± 0.021	0.21 ± 0.016	0.19 ± 0.041	0.20 ± 0.012	0.18 ± 0.014	0.17 ± 0.016
6	RB (%)	100	112	113	126	123	128	141
7	k_a (hr^{-1})	2.66 ± 0.28	2.69 ± 0.32^{NS}	2.70 ± 1.27^{NS}	2.76 ± 0.34^{NS}	2.74 ± 0.18^{NS}	2.77 ± 0.42	2.81 ± 1.10^{NS}

Values are in mean ± SEM; n=6 in each group; P<0.05 in comparison with control; C_{max}: peak concentration; t_{max}: time to reach peak concentration; AUC: area under plasma concentration time curve from 0 hrs to 24 hr; $t_{1/2}$: plasma half life; k_{el}: elimination rate constant; RB (%): relative bioavailability; ka: absorption rate constant; NS: not significant

5.3.2. Effects of *H.nepalense* root extract and compound II on bioavailability and pharmacokinetics of antibiotics.

The validated HPLC assay methods were applied to determine the plasma concentration of antibiotics (discussed in **5.3.1**) in rabbit. The plasma concentration of amoxycillin after oral administration of amoxycillin co-administered and administered 30 min after the administration of methanol root extract and compound II are shown in Table 5.13. The data fitted to a one compartment open model, which followed first order kinetics. The bioavailability and the pharmacokinetic parameters of amoxycillin after co-administration and preadministered with methanol root extract and compound II are shown in Table 5.14. In all types of the administration absorption process was complete within median t_{max} of 1.5 hr.

When amoxycillin (100 mg/kg) were co-administered or administered 30 min after the administration of the root extract, C_{max} of amoxycillin were failed to show any significant differences when compared with the control. The $t_{1/2}$ of amoxycillin after both types of the administration of extract were remained unchanged as 1.5 hr. Comparison of the other pharmacokinetic parameters failed to show any differences in elimination rate constant (k_{el}), percentage of relative bioavailability (RB %) and area under plasma concentration-time curve (AUC). The absorption rate constants (k_a) were within 2.32 (hr^{-1}) compared to 2.31 (hr^{-1}) of the control group. After the co-administration of amoxycillin with compound II at 25 and 50 mg/kg body weight, C_{max} of amoxycillin were increased significantly ($P<0.05$) to 6.14 ± 0.42 μg/ml and 6.97 ± 0.29 μg/ml respectively compared with 5.27 ± 0.22 μg/ml for the control. However, the t_{max} were remained unchanged in the entire groups. When compound II was preadministered 30 min before the administration of amoxycillin at the same dose of 25 and 50 mg/kg body weight the C_{max} of amoxycillin were further increased significantly to 6.13 ± 0.37 μg/ml and 6.96 ± 0.22 μg/ml respectively with unchanged t_{max}. In addition, the co-administration and preadministration of compound II at 50 mg/kg body weight with amoxycillin has prolonged the plasma half-life ($t_{1/2}$) and increased the area under curve (AUC) compared to the control. The relative bioavailability of amoxycillin administered 30 min after the administration of compound II at 50 mg/kg (181 %) was higher than co-administration of

the same dose of compound II (164 %). The absorption rate constant (k_a) was increased but was not statistically significant.

The result outlined in Table 5.15 shows mean plasma concentration versus time profile of cefixime administered alone and in combination with methanol root extract and isolated compound II. The bioavailability and the pharmacokinetic parameters of cefixime after co-administration or preadministration with compound II are shown in Table 5.16. In all types of the administration the absorption process was complete with unchanged median t_{max} of 1.5 hr. The t_{max} was not statistically significant ($P>0.05$). The distribution phase was short in all the groups, a fall of concentration of the antibiotic being evident within 2 hr of its administration. Comparison of other pharmacokinetic parameters of the coadministered and preadministered groups with the control group failed to show any differences either in peak drug levels (C_{max}) or mean elimination half-life ($t_{1/2}$) attained. There is no significant difference between the respective AUC values. But the group in which compound II was administered 30 min before administration of amoxycillin significantly increased the AUC value to 17.03 ± 2.15 μg/ml compared with 14.73 ± 3.58 μg/ml of the control group. The absorption rate constant (k_a) and relative bioavailability (RB %) was increased in the preadministered groups with compound II in a dose dependent manner. The absorption rate constant (k_a) was increased but was not statistically significant.

The plasma concentrations of rifampicin after oral administration of the rifampicin administered with or without methanol root extract and compound II is outlined in Table 5.17. The data fitted to a one compartment open model which followed first order kinetics and other pharmacokinetic pattern derived from these data are summarized in Table 5.18. In all types of the administration absorption process was complete within median t_{max} of 2.5 hr. When rifampicin (100 mg/kg) co-administered and preadministered with root extract, C_{max} of rifampicin was increased up to 7.11 ± 0.25 μg/ml and 7.14 ± 0.43 μg/ml compared to control where it was observed to be 6.95 ± 0.34 μg/ml. On the other hand, the t_{max} of both the group was remained unchanged as 2.5 hr as in the control group. Comparison of other pharmacokinetic parameters of the coadministered and

preadministered groups with the control group also failed to show any differences in the elimination half-life ($t_{1/2}$), elimination rate constant (k_{el}), area under curve (AUC) and relative bioavailability (RB %). There was also no significant difference in the absorption rates constant (k_a) of the groups with the control. After co-administration of rifampicin with compound II at 25 and 50 mg/kg body weights, C_{max} of rifampicin was increased significantly ($P<0.05$) to 7.93 ± 0.18 μg/ml and 8.96 ± 0.62 μg/ml respectively. In addition, the t_{max} was attained 0.5 hr sooner than the t_{max} of the control group (2.0 versus 2.5 hr). The AUC of rifampicin was significantly increased in both the co-administered and preadministered groups up to 54 43 ± 4.61 μg.hr/ml and 65.33 ± 2.36 μg.hr/ml respectively in a dose dependent manner compared to 43.43 ± 3.49 μg.hr/ml for the control group. The half-life ($t_{1/2}$) of rifampicin in which it was co-administered with compound II was prolonged significantly (4.07 ± 0.7 hr) compared to the control group (3.01 ± 0.42 hr). The absorption rate constant (k_a) was increased but not statistically significant. In the same way, the groups in which compound II at 25 and 50 mg/kg body weight was preadministered 30 mins before the administration of rifampicin, C_{max} of rifampicin was further increased significantly to 8.14 ± 0.34 μg/ml and 9.30 ± 0.20 μg/ml respectively compared to the control group (6.95 ± 0.34) μg/ml with reduction of t_{max} to 1.5 ± 0.46 hr & 1.5 ± 0.18 hr respectively compared to control group (2.5 ± 0.27 hr). The AUC value of rifampicin was increased significantly in the entire groups compared to the control group. Comparison of other pharmacokinetic parameters showed significant difference in half-life ($t_{1/2}$) and elimination rate constant (k_{el}). The relative bioavailability of rifampicin administered 30 min before the administration of compound II at 25 and 50 mg/kg body weight was found to be 129 and 165% compared to 124 and 149% for co-administration of the same doses of compound II with rifampicin. The absorption rate constants (k_a) was increased in all the groups compared to that of control group but not statistically significant.

Table 5.13 Mean plasma concentration of amoxycillin after oral administration of amoxycillin (100 mg/kg), co-administration with methanol extract of *H.nepalense* (550 mg/kg) and compound II (25, 50 mg/kg) and preadministered with *H.nepalense* (550 mg/kg) and compound II (25, 50 mg/kg) in rabbit.

Sl. No	Time of collection (hr)	(Group I) Amoxycillin (100 mg/kg)	(Group II) methanol extract (550 mg/kg) + amoxycillin (100 mg/kg)	(Group III) Compound II (25 mg/kg) + amoxycillin (100 mg/kg)	(Group IV) Compound II (50 mg/kg) + amoxycillin (100 mg/kg)	(Group V) methanol extract (550 mg/kg) + amoxycillin (100 mg/kg) 30 min later	(Group VI) Compound II (25 mg/kg) + amoxycillin (100 mg/kg) 30 min later	(Group VII) Compound I (50 mg/kg) + amoxycillin (100 mg/kg) 30 min later
1	0.5	1.78 ± 0.161	1.74 ± 0.322	2.23 ± 0.256	3.17 ± 0.347	1.78 ± 0.362	2.26 ± 0.414	3.22 ± 0.351
2	1.0	3.16 ± 0.245	3.17 ± 0.361	4.37 ± 0.228	5.12 ± 0.235	3.19 ± 0.452	4.41 ± 0.234	5.14 ± 0.228
3	1.5	5.27 ± 0.227	5.30 ± 0.234	6.14 ± 0.426	6.97 ± 0.291	5.34 ± 0.712	6.13 ± 0.378	6.96 ± 0.227
4	2.0	4.24 ± 0.172	4.28 ± 0.612	5.32 ± 0.128	5.88 ± 0.243	4.27 ± 0.152	5.31 ± 0.196	5.89 ± 0.128
5	2.5	3.58 ± 0.261	3.52 ± 0.277	4.19 ± 0.147	4.93 ± 0.329	3.54 ± 0.423	4.18 ± 0.434	4.97 ± 0.426
6	3.0	2.34 ± 0.231	2.36 ± 0.264	3.37 ± 0.135	3.86 ± 0.521	2.41 ± 0.312	3.38 ± 0.619	3.84 ± 0.239
7	4.0	1.36 ± 0.418	1.39 ± 0.411	2.62 ± 0.342	3.02 ± 0.246	1.36 ± 0. 311	2.64 ± 0.512	3.07 ± 0.432
8	5.0	1.11 ± 0.451	1.12 ± 0.381	1.47 ± 0.521	2.18 ± 0.438	1.18 ± 0.464	1.44 ± 0.247	2.14 ± 0.272
9	6.0	0.95 ± 0.026	0.96 ± 0.024	1.02 ± 0.115	1.62 ± 0.252	0.99 ± 0.023	1.04 ± 0.187	1.61 ± 0.256
10	8.0	0.51 ± 0.047	0.53 ± 0.052	0.94 ± 0.042	1.03 ± 0.032	0.61 ± 0.013	0.96 ± 0.162	1.04 ± 0.126
11	10.0	0.32 ± 0.024	0.33 ± 0.012	0.43 ± 0.052	0.64 ± 0.036	0.32 ± 0.026	0.44 ± 0.052	0.65 ± 0.042
12	12.0	0.14 ± 0.043	0.15 ± 0.012	0.29 ± 0.021	0.31 ± 0.022	0.16 ± 0.021	0.31 ± 0.018	0.34 ± 0.013
13	24.0	0.08 ± 0.009	0.09 ± 0.006	0.12 ± 0.015	0.16 ± 0.034	0.10 ± 0.014	0.14 ± 0.012	0.15 ± 0.021

Values are expressed in μg/ml, and mean ± SEM: n=6 in each group. $P<0.05$ in comparison with control

Table 5.14 Comparison of pharmacokinetic parameters of amoxycillin alone and in combination with methanol extract of *H.nepalense* and compound II

Sl.No	Parameters	(Group I) Amoxycillin (100 mg/kg)	(Group II) methanol extract (550 mg/kg) + amoxycillin (100 mg/kg)	(Group III) Compound II (25 mg/kg) + amoxycillin (100 mg/kg)	(Group IV) Compound II (50 mg/kg) + amoxycillin (100 mg/kg)	(Group V) methanol extract (550 mg/kg) + amoxycillin (100 mg/kg) 30 min later	(Group VI) Compound II (25 mg/kg) + amoxycillin (100 mg/kg) 30 min later	(Group VII) Compound I (50 mg/kg) + amoxycillin (100 mg/kg) 30 min later
1	C_{max} (µg/ml)	5.27 ± 0.22	5.30 ± 0.23^{NS}	6.14 ± 0.42^{NS}	6.97 ± 0.29	5.34 ± 0.71	6.13 ± 0.37	6.96 ± 0.22
2	t_{max} (hr)	1.5 ± 0.32	1.5 ± 0.36^{NS}	1.5 ± 0.29^{NS}	1.5 ± 0.42^{NS}	1.5 ± 0.54^{NS}	1.5 ± 0.22^{NS}	1.5 ± 0.28^{NS}
3	AUC (µg.hr/ml)	17.75 ± 2.29	18.0 ± 3.62^{NS}	24.67 ± 2.54	29.17 ± 4.32	18.44 ± 2.46	25.11 ± 1.23	30.22 ± 3.54^{NS}
4	$t_{1/2}$ (hr)	1.5 ± 0.21	1.5 ± 0.36	1.9 ± 0.18	2.3 ± 0.46	1.5 ± 0.25	2.0 ± 0.32	2.4 ± 0.29
5	k_{el} (hr^{-1})	0.46 ± 0.028	0.46 ± 0.032^{NS}	0.36 ± 0.027	0.30 ± 0.032	0.46 ± 0.034	0.34 ± 0.018	0.28 ± 0.016
6	RB (%)	100	101	139	164	103	141	181
7	k_a (hr^{-1})	2.31 ± 0.12	2.29 ± 0.87^{NS}	2.47 ± 0.23^{NS}	2.78 ± 0.74^{NS}	2.32 ± 0.42^{NS}	2.48 ± 1.10^{NS}	2.94 ± 0.47^{NS}

Values are in mean ± SEM; n=6 in each group; P<0.05 in comparison with control; C_{max}: peak concentration; t_{max}: time to reach peak concentration; AUC: area under plasma concentration time curve from 0 hrs to 24 hr; $t_{1/2}$: plasma half life; k_{el}: elimination rate constant; RB (%): relative bioavailability; ka: absorption rate constant; NS: not significant.

Table 5.15 Mean plasma concentration of cefixime after oral administration of cefixime (100 mg/kg), co-administration with methanol extract of *H.nepalense* (550 mg/kg) and compound II (25, 50 mg/kg) and preadministered with *H.nepalense* (550 mg/kg) and compound II (25, 50 mg/kg) in rabbit.

Sl. No	Time of collection (hr)	(Group I) Cefixime (100 mg/kg)	(Group II) methanol extract (550 mg/kg) + cefixime (100 mg/kg)	(Group III) Compound II (25 mg/kg) + cefixime (100 mg/kg)	(Group IV) Compound II (50 mg/kg) + cefixime (100 mg/kg)	(Group V) methanol extract (550 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VI) Compound II (25 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VII) Compound II (50 mg/kg) + cefixime (100 mg/kg) 30 min later
1	0.5	1.76 ± 0.218	1.81 ± 0.241	1.74 ± 0.365	1.76 ± 0.374	1.79 ± 0.435	1.79 ± 0.423	1.92 ± 0.423
2	1.0	2.97 ± 0.165	2.94 ± 0.235	2.90 ± 0.263	2.95 ± 0.371	2.91 ± 0.262	3.02 ± 0.233	2.99 ± 0.327
3	1.5	4.03 ± 0.428	4.04 ± 0.247	4.02 ± 0.427	4.11 ± 0.271	4.08 ± 0.264	4.24 ± 0.325	4.14 ± 0.356
4	2.0	3.54 ± 0.479	3.56 ± 0.238	3.58 ± 0.395	3.58 ± 0.503	3.57 ± 0.824	3.63 ± 0.128	3.67 ± 0.473
5	2.5	2.81 ± 0.597	2.79 ± 0.361	2.78 ± 0.223	2.79 ± 0.360	2.76 ± 0.172	2.84 ± 0.253	2.83 ± 0.763
6	3.0	2.09 ± 0.332	2.07 ± 0.321	2.22 ± 0.218	2.16 ± 0.147	2.11 ± 0.322	2.28 ± 0.423	2.24 ± 0.536
7	4.0	1.82 ± 0.451	1.87 ± 0.483	1.84 ± 0.436	1.79 ± 0.261	1.81 ± 0.328	1.81 ± 0.522	1.87 ± 0.345
8	5.0	1.10 ± 0.263	1.11 ± 0.321	1.11 ± 0.451	1.13 ± 0.156	1.12 ± 0.491	1.22 ± 0.024	1.23 ± 0.175
9	6.0	0.86 ± 0.033	0.91 ± 0.332	0.79 ± 0.034	0.84 ± 0.036	0.82 ± 0.064	0.86 ± 0.063	0.94 ± 0.026
10	8.0	0.42 ± 0.014	0.44 ± 0.625	0.41 ± 0.063	0.42 ± 0.025	0.43 ± 0.035	0.44 ± 0.032	0.49 ± 0.018
11	10.0	0.17 ± 0.021	0.16 ± 0.032	0.19 ± 0.062	0.21 ± 0.013	0.18 ± 0.054	0.23 ± 0.058	0.23 ± 0.015
12	12.0	0.08 ± 0.065	0.09 ± 0.004	0.08 ± 0.003	0.11± 0.012	0.09 ± 0.005	0.14 ± 0.046	0.16 ± 0.012
13	24.0	0.02 ± 0.004	0.01 ± 0.005	0.01 ± 0.005	0.01 ± 0.006	0.02 ± 0.003	0.05 ± 0.008	0.09 ± 0.005

Values are expressed in μg/ml and mean ± SEM; n=6 in each group. $P<0.05$ in comparison with control

Table 5.16 Comparison of pharmacokinetic parameters of cefixime alone and in combination with methanol extract of *H.nepalense* and compound II

Sl. No	Parameters	(Group I) Cefixime (100 mg/kg)	(Group II) methanol extract (550 mg/kg) + cefixime (100 mg/kg)	(Group III) Compound II (25 mg/kg) + cefixime (100 mg/kg)	(Group IV) Compound II (50 mg/kg) + cefixime (100 mg/kg)	(Group V) methanol extract (550 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VI) Compound II (25 mg/kg) + cefixime (100 mg/kg) 30 min later	(Group VII) Compound II (50 mg/kg) + cefixime (100 mg/kg) 30 min later
1	C_{max} (µg/ml)	4.03 ± 0.42	4.04 ± 0.24	4.02 ± 0.42	4.11 ± 0.27	4.08 ± 0.26	4.24 ± 0.32	4.14 ± 0.35
2	t_{max} (hr)	1.5 ± 0.22	1.5 ± 0.31 ^{NS}	1.5 ± 0.27 ^{NS}	1.5 ± 0.38 ^{NS}	1.5 ± 0.17 ^{NS}	1.5 ± 0. 57 ^{NS}	1.5 ± 0.46 ^{NS}
3	AUC (µg.hr/ml)	14.73 ± 3.58	15.33 ± 1.92^{NS}	15.12 ± 4.68^{NS}	15.61 ± 3.34 ^{NS}	15.23 ± 2.37^{NS}	16.37 ± 4.12	17.03 ± 2.15
4	$t_{1/2}$ (hr)	3.01 ± 0.21	3.01 ± 0.35	3.00 ± 0.14	3.02 ± 0.56	3.01 ± 0.22	3.11 ± 0.61	3.2 ± 0.15
5	k_{el} (hr^{-1})	0.23 ± 0.046	0.23 ± 0.024	0.23 ± 0.018	0.22 ± 0.032	0.23 ± 0.015	0.22 ± 0.016	0.21 ± 0.018
6	RB (%)	100	104	102	105	103	111	115
7	k_a (hr^{-1})	2.31 ± 0.12	2.31 ± 0.67^{NS}	2.31 ± 0.19	2.34 ± 0.42^{NS}	2.33 ± 0.38^{NS}	2.38 ± 1.15^{NS}	2.40 ± 0.28^{NS}

Values are in mean ± SEM; n=6 in each group; P<0.05 in comparison with control; C_{max}: peak concentration; t_{max}: time to reach peak concentration; AUC: area under plasma concentration time curve from 0 hrs to 24 hr; $t_{1/2}$: plasma half life; k_{el}: elimination rate constant; RB (%): relative bioavailability; ka: absorption rate constant; NS: not significant.

Table 5.17 Mean plasma concentration of rifampicin after oral administration of rifampicin (100 mg/kg), co-administration with methanol extract of *H.nepalense* (550 mg/kg) and compound II (25. 50 mg/kg) and preadministered with *H.nepalense* (550 mg/kg) and compound II (25, 50 mg/kg) in rabbit.

Sl. No	Time of collection (hr)	(Group I) Rifampicin (100 mg/kg)	(Group II) methanol extract (550 mg/kg) + rifampicin (100 mg/kg)	(Group III) Compound II (25 mg/kg) + rifampicin (100 mg/kg)	(Group IV) Compound II (50 mg/kg) + rifampicin (100 mg/kg)	(Group V) methanol extract (550 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VI) Compound II (25 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VII) Compound II (50 mg/kg) + rifampicin (100 mg/kg) 30 min later
1	0.5	2.68 ± 0.236	2.61 ± 0.335	3.12 ± 0.289	3.64 ± 0.747	2.64 ± 0.321	3.26 ± 0.411	3.82 ± 0.346
2	1.0	3.49 ± 0.424	3.58 ± 0.282	3.97 ± 0.158	4.12 ± 0.224	3.57 ± 0.136	4.04 ± 0.269	5.87 ± 0.462
3	1.5	4.08 ± 0.351	4.54 ± 0.721	5.26 ± 0.462	5.89 ± 0.251	4.56 ± 0.342	8.14 ± 0.376	9.23 ± 0.204
4	2.0	5.81 ± 0.365	6.12 ± 0.147	7.93 ± 0.182	8.96 ± 0.623	6.16 ± 0.223	7.79 ± 0.661	8.86 ± 0.262
5	2.5	6.95 ± 0.342	7.11 ± 0.258	7.17 ± 0.621	8.02 ± 0.536	7.14 ± 0.431	7.21 ± 0.492	8.08 ± 0.213
6	3.0	6.16 ± 0.427	6.38 ± 0.225	6.69 ± 0.548	7.78 ± 0.542	6.32 ± 0.174	6.69 ± 0.324	7.79 ± 0.425
7	4.0	5.64 ± 0.439	5.79 ± 0.237	5.83 ± 0.364	6.97 ± 0.254	5.69 ± 0.245	5.92 ± 0.352	6.96 ± 0.420
8	5.0	4.68 ± 0.157	4.86 ± 0.169	4.78 ± 0.525	5.83 ± 0.424	4.93 ± 0.364	4.76 ± 0.313	5.87 ± 0.413
9	6.0	3.52 ± 0.236	3.67 ± 0.493	3.92 ± 0.264	4.92 ± 0.668	3.65 ± 0.274	3.89 ± 0.621	4.94 ± 0.815
10	8.0	2.36 ± 0.428	2.42 ± 0.221	2.86 ± 0.671	3.76 ± 0.446	2.48 ± 0.245	2.91 ± 0.121	3.78 ± 0.309
11	10.0	1.05 ± 0.412	1.23 ± 0.109	1.98 ± 0.282	2.52 ± 0.462	1.25 ± 0.163	1.97 ± 0.018	2.34 ± 0.386
12	12.0	0.66 ± 0.017	0.76 ± 0.021	1.14 ± 0.018	1.32 ± 0.024	0.79 ± 0.011	1.22 ± 0.035	1.86 ± 0.252
13	24.0	0.12 ± 0.032	0.19 ± 0.011	0.26 ± 0.025	0.37 ± 0.026	0.18 ± 0.023	0.25 ± 0.016	0.52 ± 0.018

Values are expressed in μg/ml and mean ± SEM; n=6 in each group. $P<0.05$ in comparison with control

Table 5.18 Comparison of pharmacokinetic parameters of rifampicin alone and in combination with methanol extract of *H.nepalense* and compound II

Sl. No	Parameters	(Group I) Rifampicin (100 mg/kg)	(Group II) methanol extract (550 mg/kg) + rifampicin (100 mg/kg)	(Group III) Compound II (25 mg/kg) + rifampicin (100 mg/kg)	(Group IV) Compound II (50 mg/kg) + rifampicin (100 mg/kg)	(Group V) methanol extract *H.nepalense* (550 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VI) Compound II (25 mg/kg) + rifampicin (100 mg/kg) 30 min later	(Group VII) Compound II (50 mg/kg) + rifampicin (100 mg/kg) 30 min later
1	C_{max} (µg/ml)	6.95 ± 0.34	7.11 ± 0.25	7.93 ± 0.18	8.96 ± 0.62	7.14 ± 0.43	8.14 ± 0.37	9.23 ± 0.20
2	t_{max} (hr)	2.5 ± 0.27	2.5 ± 0.32 ^{NS}	2.0 ± 0.24	2.0 ± 0.23	2.5 ± 0.31 ^{NS}	1.5 ± 0.46	1.5 ± 0.18
3	AUC (µg.hr/ml)	43.93 ± 3.49	46.66 ± 3.25^{NS}	54.43 ± 4.61	65.33 ± 2.36	46.9 ± 2.36	56.52 ± 3.18	72.35 ± 3.62^{NS}
4	$t_{1/2}$ (hr)	3.01 ± 0.42	3.01 ± 0.32	3.62 ± 0.42	3.85 ± 0.42	3.01 ± 0.16	3.64 ± 0.31	4.07 ± 0.78
5	k_{el} (hr $^{-1}$)	0.23 ± 0.024	0.23 ± 0.018	0.19 ± 0.016	0.18 ± 0.041	0.23 ± 0.015	0.19 ± 0.021	0.17 ± 0.013
6	RB (%)	100	106	124	149	107	129	165
7	k_a (hr $^{-1}$)	2.66 ± 0.22	2.68 ± 0.46^{NS}	2.73 ± 1.27 ^{NS}	2.86 ± 0.34^{NS}	2.68 ± 0.18^{NS}	2.74 ± 0.76^{NS}	2.89 ± 0.37^{NS}

Values are mean ± SEM; n=6 in each group; P<0.05 in comparison with control; C_{max}: peak concentration; t_{max}: time to reach peak concentration; AUC: area under plasma concentration time curve from 0 hrs to 24 hr; $t_{1/2}$: plasma half life; k_{el}: elimination rate constant; RB (%): relative bioavailability; ka: absorption rate constant; NS: not significant.

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