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Research Article

PHARMACOKINETIC STUDIES OF AMBROXOL HYDROCHLORIDE MICROSPHERES IN RATS AFTER ORAL ADMINISTRATION

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ABSTRACT

To administer drug optimally, knowledge is needed not only of the mechanism of drug absorption, distribution and elimination but also of the kinetics of these processes, that is, pharmacokinetics. The application of pharmacokinetic principles of the therapeutic management of patients is *clinical pharmacokinetics*[1]. Pharmacokinetics and biotransformation of ambroxol chemically trans-4-(2-amino-3, 5-dibromo-benzylamino) cyclohexanol hydrochloride was studied by using increasing the number of rats or groups of rats. Absorption after oral administration was found to be fast and complete. Pharmacokinetic parameters in the blood were estimated by Standard chromatogram of Ambroxol by HPLC with electrochemical detector as 20--25 hrs in rat by statistical analysis. Ambroxol is an active substance with a long history that influences parameters considered to be the basis for the physiological production and the transport of the bronchial mucus. Therefore, ambroxol's indication is secretolytic therapy in acute and chronic bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. t½ calculated in groups I and II shows a standard deviation of 0.2 and standard error 0.141, whereas in group I, II and III it shows standard error 0.104 which shows that increasing the groups or number of rats to determine pharmacokinetic parameters minimize the error in determining particular value and make the observations more precise. All the pharmacokinetic parameters calculated from group I, II and III shows low standard error value than the pharmacokinetic calculated from group I and II as shown in Table 9 & 10. Probability or confidence of findings a true value of particular pharmacokinetic parameters increases by increasing the number of groups or number of observation. As shown in Table 11 & 12. The confidence limit reduces by increasing the number of groups or number of observation, which means that the range in which true value lies become shorten and therefore the true value of particular pharmacokinetic parameter is obtained by increasing the number of groups or rats.

Keywords: Ambroxol HCl, Microspheres, Pharmacokinetic studies, Rats.

INTRODUCTION

Ambroxol, the most frequently used mucolytic 7, 9 agent in clinical practice, affects both ciliated and secretory cells in the respiratory system. It stimulates ciliary activity as well as incorporation of precursors into phospholipids in granular pneumocytes causing thus a decrement of mucus adhesion to the hypo phase. According to pharmacological studies, it facilitates incorporation of hydrolytic

enzymes into lysosomes of the airways' secretory cells. Activation of these acidic mucopolysaccharide-degrading enzymes leads to a decrease of the sputum viscosity
Pharmacokinetics 8
Absorption, Distribution, Metabolism, and Excretion
Under peroral administration it is quickly and fully absorbed and penetrates well into the lung's tissue. Maximal concentration in blood plasma is achieved in 2 hours after administration of the medicinal product. It was found that after a single 30 mg oral dose of ambroxol, the mean Peak plasma concentration was 88.8 ng/ml. Bioavailability of ambroxol administered orally is approximately 70–80%. The distribution half-life of ambroxol is 1.3 hours, metabolite is dibromoanthranilic acid (activity unspecified) and Elimination of ambroxol is biphasic, with an alpha half-life of 1.3 hours and a beta half-life of 8.8 hours. Excretion is primarily by the kidneys, Renal clearance (rate) is approximately 53 ml/minute; approximately 5–6% of a dose is excreted unchanged in the urine. The elimination half-life of the parent compound is 8.8 hours.

MATERIALS AND METHODS

Ambroxol (Jackson labs (p) ltd. Punjab), UV/VIS Spectrophotometer Systronics, Cooling Centrifuge Remi model C24, HPLC with Electro Chemical Detector, Remi sales & Engineering ltd.

METHODS

IDENTIFICATION STUDYS OF AMBROXOL IR ANALYSIS [2, 3].

IR spectra of Ambroxol are obtained with FT/IR–4100 type a spectrophotometer using the potassium bromide disk (KBr) technique.

UV SPECTROPHOTOMETER

Dilute 2.0 mg of Ambroxol in 0.05M sulphuric acid and dilute to 100.0 ml with the same acid. Dilute 2.0 ml of the solution to 10.0 ml with 0.05 M sulphuric acid. Examined between 200nm and 350nm, the solution shows two absorption maxima at 245 nm and 310 nm. The ratio of the absorbance measured at 245 nm to that measured at 310 nm is 3.2 to 3.4.

STANDARD CHROMATOGRAM OF AMBROXOL BY HPLC WITH ELECTROCHEMICAL DETECTOR

Prepared stock solution of Ambroxol 100mg/100ml in mobile phase (50mM phosphate buffer and methanol, 3:7 v/v). From this stock solution, concentrations that include 1ng/ml were prepared in mobile phase and 60µl of the solution is injected into the HPLC with electro chemical detector monitored at 850 mV to obtain a standard chromatogram.

HPLC ASSAY OF AMBROXOL[4]

The concentrations of Ambroxol in plasma were analyzed by the reported HPLC method. 1 ml of 25mM borate buffer were added to the plasma sample in glass test tube. After vigorous mixing, 6 ml of diethyl ether was added and mixed for 15 mins using cyclomixer. After centrifugation for 15 mins at 3000 rpm, organic phase was transferred to clean glass tube and evaporated under slight nitrogen stream. The residue was reconstituted with 200µl of n-heptane and then back extracted using 200µl of 0.01M hydrochloride. A 70µl of aqueous phase was transferred to clean glass tube, and 70 µl of mobile phase was added. A 60 µl of the mixture was injected on to the HPLC column. The mobile phase, a mixture of 50mM phosphate buffer (50 mM potassium phosphate; monobasic: 50 mM potassium phosphate; dibasic = 6:4) and methanol (3:7 v/v) was run at a flow rate of 0.2 ml per minute.

CHROMATOGRAPHIC CONDITIONS

Column : Capcell Pac C18 MG-Shiseido
Mobile phase: 50mM phosphate buffer and methanol (3:7 v/v)
Flow rate: 0.2ml/min
Detection: elector chemical detection at 850 mv
Temperature: 25oC
Detector: HPLC
Pressure: 36kgf

DRUG ADMINISTRATION AND SAMPLE COLLECTION

Pharmacokinetic evaluation was performed in Wister rats of either sex and of approximately the same age, weighing 175 – 225 gm were used for the study. They were housed in polypropylene cages and fed with standard Chow diet and water ad libitum. The animals are exposed to an alternate cycle of 12 hours of darkness and light each. Before the test the animal were fasted for at least 12hours.
Three groups of eleven rats were used. Animals were administered 2.7 mg/kg of Ambroxol Hcl through oral route after overnight fasting. The blood samples were collected at different time intervals at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hour after the administration of drug. The heparinized normal saline solution 1ml was flushed after each blood sampling. The blood sample was centrifuged immediately and the plasma was separated. Concentrations were obtained from the standard chromatogram.

DETERMINATION OF PHARMACOKINETIC PARAMETERS[5,10]

Cmax

Peak plasma level computed directly from the plasma level profiles.

Tmax

Time to achieve the peak plasma level computed directly from the plasma level profiles.

Kel

Elimination rate constant is calculated from the terminal elimination phase of log plasma concentration Vs time by least square regression analysis. From the slope of regression analysis.

Kel is calculated as

Kel = 2.303 x slope

Biological Half Life

t ½ = 0.693/Kel

AUC 0→t*

The extent of absorption is calculated from the area under the plasma concentration time curve from O to t* hours by trapezoidal rule method.

Trapezoidal rule AUC =

$$\int_{t1}^{t2} \frac{(C_1 - C_2)(t_2 - t_1)}{\ln C_1 - \ln C_2}$$

AUC 0→∞

The estimation of area under the blood level time curve from zero time to infinity must be carried out in 2 steps.

The area under the curve from zero to time t* is calculated by means of trapezoidal rule.

The area under curve from t* to ∞ is calculated by using formula.

AUC t*→∞ = C*/λn

Where λn = 2.303 times the slope of the terminal exponential phase of a plot of log conc. Vs time.

T* = time at which blood sampling is stopped

C* = concentration of drug at t*

AUMC0→t*

Plot of the product of concentration and time Vs time from 0 time to t* is often referred to as the area under the first moments curve AUMC 0→t*

The area under the curve from 0 to t* is calculated by means of trapezoidal rule method.

AUMC0→∞

The estimation of area under the first moment curve from 0→∞ must be carried out in 2 steps.

i. The area under the curve from 0 to t* is calculated by means of trapezoidal rule.

ii. ii. The area under the curve from t* to ∞ is calculated by using the formula.

$$\text{AUMC } t^* \rightarrow \infty = \frac{t^*C^*}{n} + \frac{C^*}{n2}$$

Where

t* = Time at which blood sampling is stopped

C* = Concentration of drug at t*

Mean Residence Time (MRT)

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

COMPARISON OF PHARMACOKINETIC PARAMETERS BY STATISTICAL ANALYSIS

Standard Deviation = $\sqrt{\frac{(x-\bar{x})^2}{n-1}}$

$\bar{x}$ = arithmetic mean

x = series of observation

n = no. of observations

Coefficient of Variation (C.V) = $\frac{S \times 100}{\bar{x}}$

S = standard deviation

$\bar{x}$ = arithmetic mean

Standard Error = $\frac{S}{\sqrt{n}}$

n = no. of observations

S = standard deviation

Confidence Interval

Confidence limits of μ, for n replicate measurements

μ = $\bar{x} \pm \frac{tS}{\sqrt{n}}$

n = no. of observations

S = standard deviation

$\bar{x}$ = arithmetic mean

t is a parameter that depends upon the no. of degrees of freedom (ν) and the confidence level required.

Table 1: Chromatographic Peak Area ± Standard Deviation in different groups of rats

Time (Hours)	Peak area			Peak area (Mean ± S.D)	C.V (coefficient of variation)
	Group I	Group II	Group III		
0.5	108.1943	105.454	113.5014	109.0624 ± 3.33	3.05
1.0	512.0375	510.299	535.8123	519.3885 ± 11.63	2.24
1.5	678.5632	682.231	681.7251	680.8410 ± 1.62	0.238
2.0	750.4246	755.4542	758.8225	754.9022 ± 3.45	0.457
3.0	670.6755	675.0006	593.1449	646.2940 ± 37.6	5.82
4.0	491.2001	495.0334	497.0803	494.4384 ± 2.45	0.496
6.0	226.9312	230.1517	263.0340	240.04 ± 16.3	6.795
8.0	150.0637	151.9170	145.2837	149.0941 ± 2.79	1.875
10.0	90.3976	96.2022	95.1030	93.9126 ± 2.5	2.672
12.0	73.3671	72.0294	77.8489	75.8493 ± 2.7	3.559
24.0	32.4404	36.5362	37.1540	35.3768 ± 2.09	5.90

Table 2: Ambroxol concentration (ng/ml) in different groups of rats

Time (Hours)	Concentration (ng/ml)			Log Concentration (ng/ml)		
	Group I	Group II	Group III	Group I	Group II	Group III
0.5	3.672	3.579	3.852	0.56	0.55	0.58
1.0	17.378	17.319	18.1849	1.20	1.23	1.25
1.5	23.0297	23.1542	23.1370	1.30	1.36	1.36
2.0	25.4686	25.7536	25.6393	1.40	1.41	1.40
3.0	22.762	22.909	20.1390	1.35	1.36	1.30
4.0	16.6708	16.8009	16.8704	1.22	1.22	1.22
6.0	7.7018	7.8111	8.927	0.88	0.89	0.95
8.0	5.093	5.1559	4.9308	0.70	0.71	0.69
10.0	3.068	3.265	3.2277	0.48	0.51	0.50
12.0	2.49	2.4446	2.6421	0.39	0.38	0.42
24.0	1.1009	1.240	1.2610	0.04	0.09	0.10

Table 3: Ambroxol concentration and log concentration (ng/ml) ± standard deviation in different groups of rats

Time (hrs)	Concentration (ng/ml) (Mean ± S.D)	C.V (coefficient of variation)	Log concentration (Mean ± S.D)	C.V (coefficient of variation)
0.5	3.701 ± 0.113	3.05	0.563 ± 0.011	1.95
1.0	17.627 ± 0.039	2.24	1.220 ± 0.021	1.72
1.5	23.1069 ± 0.05	0.216	1.340 ± 0.028	2.08
2.0	25.6205 ± 0.11	0.429	1.403 ± 0.004	0.285
3.0	21.933 ± 1.27	5.79	1.336 ± 0.026	1.946
4.0	16.780 ± 0.08	0.492	1.220 ± 0.000	0.000
6.0	8.146 ± 0.55	6.75	0.900 ± 0.032	3.55
8.0	5.059 ± 0.099	1.77	0.700 ± 0.008	1.14
10.0	3.203 ± 0.086	2.49	0.496 ± 0.012	2.42
12.0	2.525 ± 0.084	3.32	0.396 ± 0.017	4.29
24.0	1.200 ± 0.071	5.91	0.076 ± 0.020	26.31

Table 4: Ambroxol concentration (ng/ml).time (hr) ± standard deviation in different groups of rats

Time (Hours)	Concentration x Time (ng/ml. Hr)			Mean (Concentration x Time) ± S.D	C.V (Coefficient of variation)
	Group I	Group II	Group III		
0.5	1.836	1.789	1.926	1.850 ± 0.057	3.08
1.0	17.378	17.319	18.1849	17.627 ± 0.39	2.21
1.5	34.54	34.73	34.70	34.66 ± 0.17	0.49
2.0	50.93	51.50	51.28	51.24 ± 0.23	0.45
3.0	68.28	68.72	60.39	65.79 ± 3.82	5.80
4.0	66.68	67.20	67.48	67.12 ± 0.33	0.49
6.0	46.26	46.87	53.56	48.89 ± 3.30	6.74
8.0	40.74	41.25	39.44	40.48 ± 0.76	1.88
10.0	30.68	32.65	32.27	31.86 ± 0.85	2.66
12.0	29.88	29.33	31.71	30.30 ± 1.01	3.33
24.0	26.42	29.76	30.26	28.81 ± 1.70	5.90

RESULTS AND DISCUSSION

IR ANALYSIS

IR spectra of ambroxol shows the peak in the range of 3500 – 2500cm -1 due to overlapping between – OH, – NH2, and > NH vibrations. Peak at 3396.99 cm -1 is observed due to primary amine and band occurred due to N=H stretching vibration. Peak at 2911.02 cm -1 occur due to methylene group due to C – H stretching peak at 1456 cm-1 is observed due to C = C stretching in aromatic moiety. Peak at 737 cm-1 show presence of ortho and Para substituted benzene. Peak at 1064.51 cm-1 occur due to secondary alcohol, alicyclic six membered ring due to C – O stretching.

UV SPECTROPHOTOMETRICALLY

Absorbance of Ambroxol at 247 nm is 0.368 and at 310 nm is 1.299 and the ratio of absorbance at 247 and 310 nm comes out to be 3.3652 which shows the identity of Ambroxol.

HPLC ASSAY OF AMBROXOL[69]

The validated HPLC assay method was applied to determine the concentration and various pharmacokinetic parameters of Ambroxol in rat plasma.

DETERMINATION OF PHARMACOKINETIC PARAMETERS

The validated HPLC assay method was applied to determine the concentration and various pharmacokinetic parameters of Ambroxol in rat plasma.

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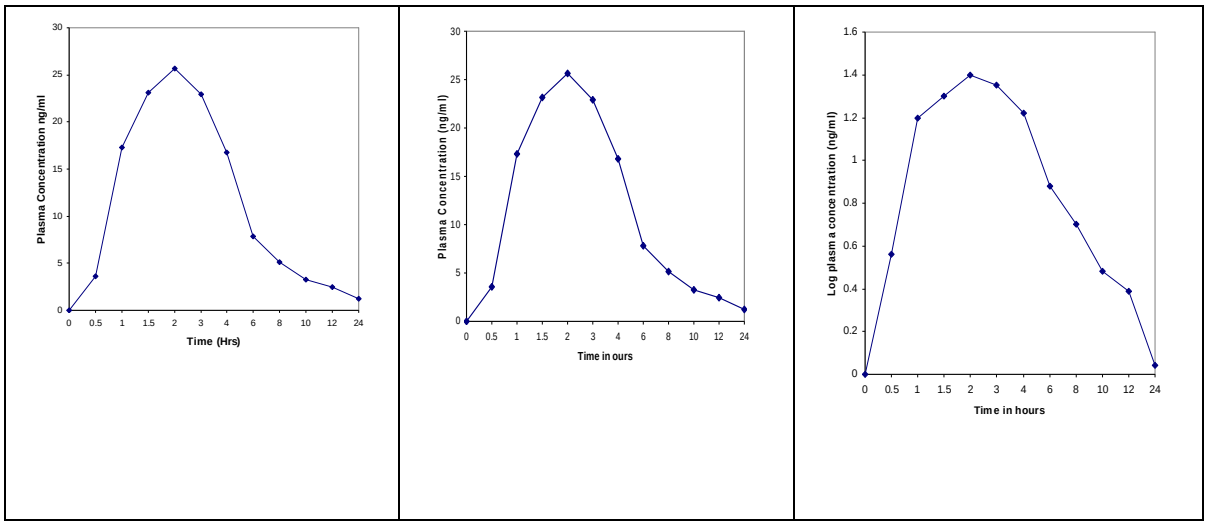

Fig. 1: Plasma concentration of Ambroxol (ng/ml) at different time intervals in Group I, II, III Rats

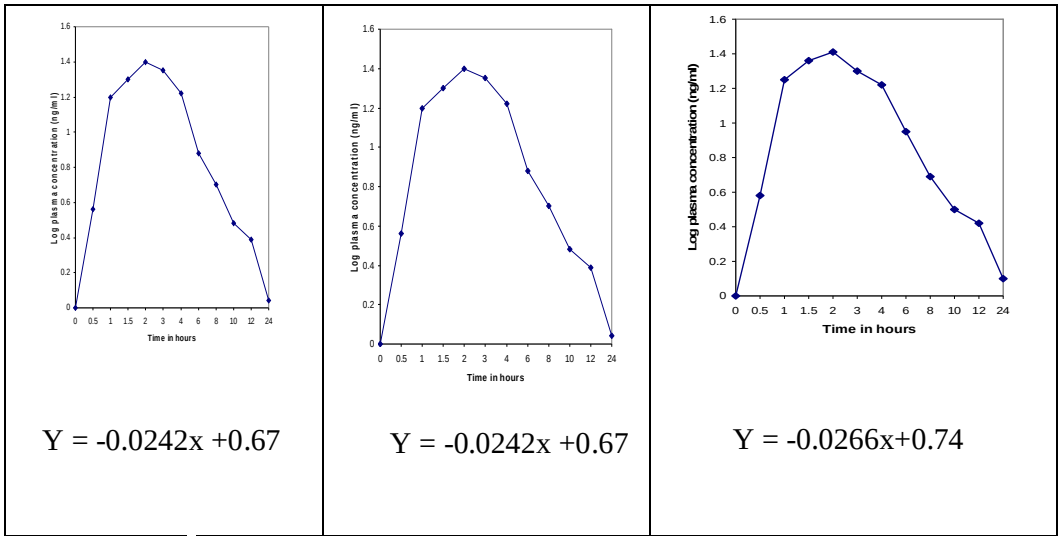

Fig. 2: Log Plasma concentration of Ambroxol (ng/ml) at different time intervals in Group I, II, III Rats

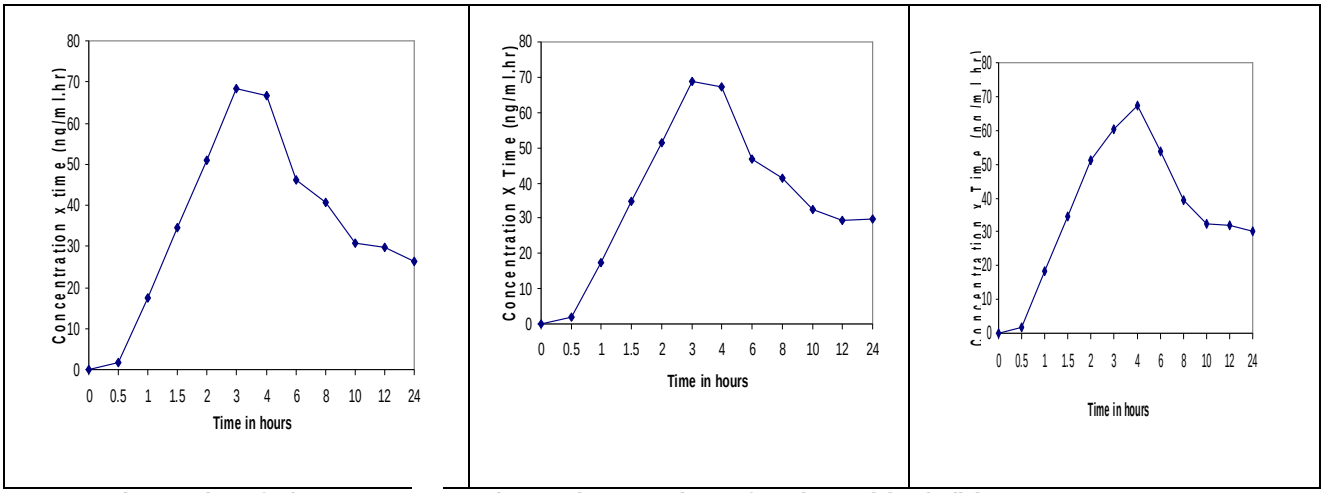

Fig. 3: Plot of Plasma concentration x Time Vs Time of Ambroxol (ng/ml) in Group I, II, III Rats

Table 5: Pharmacokinetic parameters in different groups of rats

Pharmacokinetic parameters	Group I	Group II	Group III
C_{max} (ng/ml)	25.4686	25.7536	25.6393
T_{max} (hrs.)	2.0	2.0	2.0
K_{el} (hr^{-1})	0.0671	0.0556	0.0614
$t_{½}$ (hrs)	10.3	12.4	11.2
AUC_{0-t^*} (ng.hr/ml)	137.81	142.57	143.193
$AUC_{t^*-\infty}$ (ng.hr/ml)	4.14	5.37	4.75
$AUC_{0-\infty}$ (ng.hr/ml)	141.950	147.940	147.943
$AUMC_{0-t^*}$ ($ng.hr^2$/ml)	876.75	742.82	821.16
$AUMC_{t^*-\infty}$ ($ng.hr^2$/ml)	115.05	152.22	132.04
$AUMC_{0-\infty}$ ($ng.hr^2$/ml)	991.80	895.04	953.2
MRT (hrs)	6.98	6.05	6.44

Fig 1, 2 & 3 show the plot of concentrations of Ambroxol in plasma Vs time in different groups of rats. The drug was present in detectable amount in all the three groups for 24 hours. All

the three groups show time for maximum concentration at 2.00 hour. Cmax obtained in group I, II and III was 25.4686, 25.7536 and 25.6393 ng/ml respectively and the half life of Ambroxol was calculated to be 10.3, 12.45 and 11.28 hours. The AUC for three groups was calculated to be 141.950, 147.940 and 147.943 ng.hr/ml respectively, and the area under first moment curve for three groups was calculated to be 991.80, 895.04 and 953.20 ng.hr2/ml respectively and the MRT for the three groups was calculated to be 6.98, 6.05 and 6.44 hrs. respectively and other various pharmacokinetic parameters for all the three groups is shown in Table 5. Kel (elimination rate constant) was calculated for all the three groups from the slope of terminal portion of the log concentration versus time curve with the method of Regression analysis and the equations corresponding to the regression analysis are -0.0291x+0.74, -0.0242x+0.67 and -0.0266x+0.74 respectively for the three groups and Kel (elimination rate constant) come out to be 0.06717, 0.05566 and 0.06141 hr-1 respectively for the three groups.

COMPARISON OF PHARMACOKINETIC PARAMETRES

Table-6: Pharmacokinetic parameters (Mean ± S.D) of Group I & II Rats

Pharmacokinetic parameters	Mean ± S.D	C.V (coefficient of variation)	Standard error
C_{max} (ng/ml)	25.6111 ± 0.1425	0.556	0.1
T_{max} (hrs)	2.00 ± 0.00	0.0	0.0
K_{el} (hr^{-1})	0.06141 ± 0.0175	7.04	0.0123
$t_{½}$ (hrs)	11.375 ± 0.2	7.16	0.141
AUC_{0-t^*} (ng.hr/ml)	140.19 ± 2.38	1.69	1.68
$AUC_{t^*-\infty}$ (ng.hr/ml)	4.755 ± 0.615	12.93	0.43
$AUC_{0-\infty}$ (ng.hr/ml)	144.94 ± 2.99	2.06	2.11
$AUMC_{0-t^*}$ ($ng.hr^2$/ml)	809.78 ± 66.96	8.27	47.34
$AUMC_{t^*-\infty}$ ($ng.hr^2$/ml)	133.63 ± 18.58	13.90	13.13
$AUMC_{0-\infty}$ ($ng.hr^2$/ml)	943.42 ± 48.38	5.12	34.2
MRT (hrs)	6.51 ± 0.465	7.14	0.33

Comparison of pharmacokinetic parameters between group I and II by statistical analysis is shown in Table 9. It is found that time to reach maximum concentration is same for both the groups with zero standard deviation and zero standard error, but other pharmacokinetic parameters show variation with some value of standard deviation and standard error. AUC0-∞ shows the standard deviation 2.99 and standard error 2.11, AUMC0-∞ shows the standard deviation 48.38 and standard error 34.2, Cmax shows the standard deviation 0.1425 and standard error 0.1, t ½ (half life) shows the standard deviation 0.2 and standard error 0.141, MRT shows the standard deviation 0.465 and standard error 0.33.

Table-7: Pharmacokinetic parameters (Mean ± S.D) of Group I, II & III Rats

Pharmacokinetic parameters	Mean ± S.D	C.V (coefficient of variation)	Standard error
C_{max} (ng/ml)	25.6205 ± 0.11	0.429	0.063
T_{max} (hrs)	2.00 ± 0.00	0.0	0.0
K_{el} (hr^{-1})	0.0614 ± 0.0163	6.41	0.00941
$t_{½}$ (hrs)	11.34 ± 0.18	6.59	0.104
AUC_{0-t^*} (ng.hr/ml)	141.191 ± 2.4	1.69	1.385
$AUC_{t^*-\infty}$ (ng.hr/ml)	4.75 ± 0.50	10.52	0.288
$AUC_{0-\infty}$ (ng.hr/ml)	145.944 ± 2.82	1.93	1.63
$AUMC_{0-t^*}$ ($ng.hr^2$/ml)	813.576 ± 54.9	6.74	31.69
$AUMC_{t^*-\infty}$ ($ng.hr^2$/ml)	133.103 ± 15.193	11.41	8.771
$AUMC_{0-\infty}$ ($ng.hr^2$/ml)	946.68 ± 39.77	4.20	22.96
MRT (hrs)	6.49 ± 0.38	5.85	0.219

Comparison of pharmacokinetic parameters between group I, II and III is done in Table 10. It is found that the time to reach maximum concentration is same in all the groups with zero standard deviation and zero standard error. AUC0-∞ shows the standard deviation 2.82 and standard error 1.63, AUMC0-∞ shows the standard deviation 39.77 and standard error 22.96, Cmax shows the standard deviation 0.11 and standard error

0.063, t ½ (half life) shows the standard deviation 0.18 and standard error 0.104, MRT shows the standard deviation 0.38 and standard error 0.219.

Table-8: 95% confidence level (CL) for μ of Pharmacokinetic parameters of Group I, II & III Rats

Pharmacokinetic parameters	95 % (CL) for μ (95 % confidence level for μ)
C_{max} (ng/ml)	25.6205 ± 0.273
T_{max} (hrs)	2.00 ± 0.00
K_{el} (hr^{-1})	0.0614 ± 0.04
$t_{½}$ (hrs)	11.34 ± 0.44
AUC_{0-t^*} (ng.hr/ml)	141.191 ± 5.96
$AUC_{t^*-\infty}$ (ng.hr/ml)	4.75 ± 1.24
$AUC_{0-\infty}$ (ng.hr/ml)	145.944 ± 7.0
$AUMC_{0-t^*}$ ($ng.hr^2$/ml)	813.576 ± 136.39
$AUMC_{t^*-\infty}$ ($ng.hr^2$/ml)	133.103 ± 37.74
$AUMC_{0-\infty}$ ($ng.hr^2$/ml)	946.68 ± 98.80
MRT (hrs)	6.49 ± 0.94

95% Confidence level various pharmacokinetic parameters for group I, II and III are given in Table 12. For Cmax, Kel, t ½ (half life), AUC0-∞, AUMC0-∞, MRT, 95% confidence level is 0.273, 0.04, 0.44, 7.00, 98.80, 0.94 respectively. There is 95% confidence that the true value of Cmax lies between (25.345 - 25.8935) as compared to group I and II which means the range in which the true value lies become shorten on increasing the groups of rats.

CONCLUSION

T½ calculated in groups I and II shows a standard deviation of 0.2 and standard error 0.141, whereas in group I, II and III it shows standard error 0.104 which shows that increasing the groups or number of rats to determine pharmacokinetic parameters minimize the error in determining particular value and make the observations more precise. All the pharmacokinetic parameters calculated from group I, II and III shows low standard error value than the pharmacokinetic calculated from group I and II as shown in Table 6 & 7.

Probability or confidence of findings a true value of particular pharmacokinetic parameters increases by increasing the number of groups or number of observation. As shown in Table 8. The confidence limit reduces by increasing the number of groups or number of observation, which means that the range in which true value lies become shorten and therefore the true value of particular pharmacokinetic parameter is obtained by increasing the number of groups or rats.

T½ of ambroxol is calculated to be 11.34 hr but the reported t½ of ambroxol in the literature is 3-4 hours. The resulting t½ value is due to the short sampling time schedule in this study (until 24 hr). In addition the gap between 12 and 24 hr in blood sampling schedule might cause error in evaluating terminal half life of ambroxol. Therefore there must be complete blood sampling until there is no drug detected in blood plasma in order to get the correct result. Cmax achieved is 0.254 ± 0.0163 ng/ml which shows that to detect the drug in plasma sensitive instruments like HPLC is required.

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