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ORIGINAL RESEARCH ARTICLE

Pharmacokinetics of Esomeprazole in Oryctolagus Cuniculus Male Rabbit with Immobilized Stress

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ABSTRACT

It has been reported that emotional stress influenced the drug absorption by inhibit the intestinal propulsion activity. Emotional stress is closely related to the pathogenesis of gastric ulcers. The effect of emotional stress on pharmacokinetics of antiulcer drug has not been conducted yet. Hence the present study aimed to investigate the effect of immobilization stress on pharmacokinetics of esomeprazole (gastric proton inhibitor) in Oryctolagus cuniculus male rabbits. Results showed that AUCinf, Cmax, Vz, and CL values determined without immobilized stress were higher than those determined using the immobilized stress. The bioavailability of esomeprazole without immobilized stress was found to be 85.33±21.23% which is higher than immobilized stress 61.33±19.45%, and was 1.39 fold difference($p=0.0051$, $P<0.05$). In Conclusion, Stress, in any form, can alter pharmacokinetic parameters, and thus, it is important that stress be minimized to obtained reliable pharmacokinetic data. The present study demonstrates that the immobilized stress can alters pharmacokinetics parameters significantly.

Keywords: Immobilized stress, Esomeprazole, Proton pump inhibitor, Bioavailability

INTRODUCTION

The pharmacokinetics of drugs are influenced by various factors such as age, food intake, body weight, drug interaction, and sometimes stress also [1, 2]. It has been previously reported that stress from surgery during pharmacokinetics study in small animals also influenced the drug absorption and pharmacokinetics parameters [3].

It has been reported that emotional stress influenced the drug absorption by inhibit the intestinal propulsion activity. [4] Emotional stress is closely related to the pathogenesis of gastric ulcers. [5, 6] The effect of emotional stress on pharmacokinetics of antiulcer drug has not been conducted yet. Hence the present study aimed to investigate the effect of immobilization stress on pharmacokinetics of esomeprazole (gastric proton inhibitor) in Oryctolagus cuniculus male rabbits

MATERIALS AND METHODS

Chemicals and reagents

Esomeprazole and Pantoprazole (Sigma–Aldrich Co, St Louis, MO, USA) were obtained as gift samples from department of pharmacology, college of medicine, Dankook University, Cheonan, South Korea. Methanol and acetonitrile grade were pursed from Triveni interchem P.Ltd (India). Formic acid AR was pursed from Manav Biochem Impex P.Ltd (India). Reagent grade triple deionized water was pursed from Organo Biotech Laboratories. P.Ltd (India). All other chemicals and reagents used were of analytical grade.

Apparatus and conditions

The LC-MS/MS system used was a Varian ProStar™ LC unit (Varian Inc., CA, USA) connected to a Varian 1200L quadruple. System control and data analysis were carried out using Varian MS software (Version 6.5, Varian Inc.). HPLC columns YMC® C18 (Waters, MO, USA), 50 mm × 2.0 mm, 3 µm particle size and guard column (C18, 4.0 X 2.0mm, phenomenex, CA, USA) were used for analyzing blood samples. An isocratic mobile phase consisting of solvent A (purified water containing 0.1% formic acid) and solvent B (acetronitrile containing 0.1% formic acid) mixed in the ratio of 88/12 (v/v, A/B) was

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used at a flow rate of 0.24 ml/min.The column oven was maintained at 45ºC and run time was 5 minutes. MRM (multiple reaction monitoring) transitions with collision energies (eV) for esomeprazole and pantoprazole were m/z 152.323 → 151.823 (13.5 eV) and 159.347→ 158.847 (8.0 eV), respectively. The scan time and dwell time were 0.3 sec and 0.5 sec respectively. Electrospray ionization (ESI) was performed under capillary 5000 volts, shield 600 volts, and at a temperature of 299.8°C. Manifold temperature and pressure were 42°C and 1.83 mTorr, and the detector was set at 1700 volts, fixed positive.

Animals handling and surgical procedures

2.8-3.0 kg Oryctolagus cuniculus male rabbits were pursed from College of Veterinary Sciences, Rampur, Chitwan, Nepal. The rabbits were acclimated for one week before study. Upon arrival, animals were randomized and housed one per cage in strictly controlled environmental condition of 20 to 25^{o}C temperature and 48 to 52% relative humidity (RH). A 12 hour light and dark cycle was used with an intensity of 150 to 300 Lux. All animal procedures were based on a guideline recommended by institutional animal care and experiment committee of Chitwan Medical College. Before conducting the study, rabbits were categorized into two groups; Control group (oral study, n=6 and intravenous study, n=6) and immobilized stress group (oral study, n=6).In immobilized stress group, immobilization stress was administered by restraining the rabbits in a wire net for 60 minutes immediately after administration of esomeprazole.

In both groups, the surgical procedures were carried out under Tiletamine HCl (125 mg/kg) and Zolazepam HCl (125mg/kg) anesthesia (intramuscular injection).In immobilized stress group, femoral artery cannulation was carried out for blood sampling during oral study. While in control group, femoral artery and femoral vein cannulation were carried out for blood sampling during oral and intravenous study. Considering the dead volume of catheter, a corrected dose of esomeprazole (40mg/kg) was infused through femoral vein catheter for IV study and through oral gavages for oral study. In both groups, bloods were collected from femoral artery and compensating with equal volumes of hepatinized saline. .A series of blood samples at 0, 1, 5, 15, 30, 45, 60, 90,120, 180, 240, 360, 480 720 and 1440 minutes (for IV study) and 0, 1, 5, 15, 30, 45, 60, 90,120, 180, 240, 360, 480,720 and 1440 minutes(for oral study) were collected using virtually without blood loss and compensating with equal volumes of hepatinized saline. Bioavailability were estimated by based on the AUC inf ratios from oral control group and IV control group for control group and AUC inf ratios from oral immobilization group and IV control group for immobilization stress group.

Sample preparation and validation

Blood samples-validation

The validation samples were prepared by standard working solution spiking method to access the plasma concentration of esomeprazole. For the measurement of esomeprazole in plasma sample, the validation samples were prepared by following way; an aliquot of blood plasma 90µL was spiked with 10 µl standard working solution (desirable concentration of esomperazole standard solution was prepared by dissolving appropriate amount in purified water) and 20 µl internal standard (1 µg/ml, prepared in methanol/water, 50/50 v/v), and extracted with 400 µl acetronitrile solution. The organic layer was dried under the gentle stream of nitrogen 40°C. The dried extract was reconstituted with 800 µl of 50% methanol and 5 µl was injected to LC-MS/MS system.

Lower limit of detection (LLOD) was defined as a peak with signal noise ratio(S/N) more than 10/1, while lower limit of quantification was further narrowed to have percentage coefficient of variation (CV, %) less than 15%. Five sets of validation samples at concentrations of 0.1, 0.3, 1, 2, 5, 10, 50 and 100µg/ml were used to draw calibration curve. Similarly, Inter/ Intra- day validation were assessed to validate the precision and accuracy of the assay. For interday validation, five sets of control samples at different concentrations of 0.1, 0.3, 2 and 8µg/ml were evaluated on five different days. For intraday validation, five sets of control samples at different concentrations of 0.1, 0.3, 2 and 8µg/ml with one standard curve were evaluated on same day. The assay recovery for esomperazole was assessed with five sets of quality control (QC) samples (1, 5 and 10 µg/ml) assayed randomly along with standard samples during the interday and intraday assays.

Blood samples- analysis

Sample preparation involved a protein precipitation method with acetronitrile. An aliquot of blood plasma 100µL was spiked 20 µl internal standard (1 µg/ml, prepared in methanol/water, 50/50 v/v), and extracted with 400 µl acetronitrile solution. The organic layer was dried under the gentle stream of nitrogen 40°C. The dried extract was reconstituted with 800 µl of 50% methanol and 5 µl was injected to LC-MS/MS system.

Data Analysis

Noncompartmental pharmacokinetics analysis was performed using WinNonlin™ Professional (Version 2.1, Pharsight, CA, USA).The student's *t-test* for independent data was used to compared the pharmacokinetics parameters between immobilized stress group and control group, with significance assigned at $P < 0.05$. All other data were expressed as mean± standard deviation (SD).

RESULTS AND DISCUSSION

Quantitative basis and the selection of internal Standard

In order to develop an analytical method with desired LLOD (100ng/ml), it was necessary to use MS/MS detection, because MS/MS analytical methods provide the very low limits of detection (LOD) required for trace mixture analysis.[8] The internal standard (IS) used pantoprazole (**Fig 1b**) is a structural isomer of esomeprazole (**Fig 1a**). The full scan positive mass spectra of esomeprazole and the IS produced protonated mass ions ($[M+H]^+$) at 152.32 and 159.34, respectively, in the Q1 spectrum, and these were used as precursor ions to obtain product ion spectra. Although both esomeprazole and IS have the same molecular weights, they can be individually detected due to their different fragmentation patterns. No interference was observed between esomeprazole and IS when measuring the m/z 152.32→ 151.823 transition and m/z 159.347→ 158.847 transition, respectively.

Validation

Chromatographic conditions, especially the composition of the mobile phase, were optimized to achieve good resolution and symmetrical peak shapes for esomeprazole and the IS, acceptable retention factors (k'≥2), and a short run time. The isocratic mobile phase consisting of solvent A (purified water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid) mixed in the ratio 88/12 (v/v, A/B) was found to be suitable. A flow rate of 0.24 ml/min was required to elute the esomeprazole and the IS at retention times of 1.70 and 1.71 min, respectively. The formic acid was found to be necessary in order to lower the pH and protonate esomeprazole to produce a symmetrical peak shape at a satisfactory retention factor. The percentage of formic acid was also optimized to achieve a symmetrical peak shape and good ionization and fragmentation.

The calibration curve drawn for esomeprazole in plasma for the manual method was linear over the concentration range 0.1 to 100 µg/ml. The best linear fit and least squares residuals of the calibration curve were achieved using a $1/x^2$ weighing factor, giving a mean linear regression equation for the calibration curve of $y=0.42781x + 0.002214$ where y is the peak ratio of esomeprazole to IS and x is the concentration of the esomeprazole. The correlation coefficient (r^2) for esomeprazole was 0.99991. The inter-and intra-day precisions were expressed as CV % and were below 15% (maximum 13.31% and minimum 3.25% for an LLOD sample), and the accuracy was between 82.14% and 107.19%, which complies with the FDA regulations. The recovery percentages of QC samples were between 98.45% and 105.71%. The extraction procedure showed good sensitivity, specificity, precision, accuracy, recovery, and linearity, and hence the method was successfully implemented for the analysis of blood samples (**Table 1**).

No peaks corresponding to esomeprazole or the IS were observed in blank rabbit plasma using the LC-MS/MS conditions described in (**Fig 2a**). A mass chromatogram of rabbit plasma spiked with esomeprazole and IS *was* shown in (**Fig 2b**). The mass chromatogram of blood samples at 120 min (oral study) is shown in (**Fig 3**).

Pharmacokinetics parameters without immobilized stress

The concentration–time profile of esomeprazole following its oral and intravenous administration without immobilized stress is shown in (**Fig 4a & 4b**). (**Table 2**) summarizes the pharmacokinetics parameters of esomeprazole after intravenous and oral administration, respectively. AUC_{inf} values were 3125.63±218.77 and 3612.73±208.91 for oral and intravenous administration, respectively. Esomeprazole had a short terminal half-life (404.22±22.00 and 133.41±65.92 minutes in the oral and intravenous studies, respectively) with relatively high distribution volumes during the steady and terminal phases, and with low plasma clearance. This indicates the absorption of esomeprazole is not a limiting factor for plasma clearance and extent of distribution. In the oral study, peak concentration was observed at about 120±14.27 minutes after dosing, indicating that esomeprazole was absorbed rapidly and that its absorption was independent of gastric solubility and pH. Cmax and CL values following oral administration were 57.24±3.08 and 1.97±0.14 respectively, and in the intravenous study these were 78.23±11.6, and 8.83±3.32, respectively. Bioavailability was estimated to be

85.33±21.23%based on the AUC $_{inf}$ ratios of oral and intravenous administration.

Pharmacokinetics parameters with immobilized stress

The concentration–time profile of esomeprazole following oral administration with immobilized stress is shown in (**Fig 5**). (**Table 3**) itemizes the various pharmacokinetics parameters. AUC_{inf} value was 2215.93±75.09 for oral administration. Immobolized testing also showed that esomeprazole had short terminal half-lives with relatively high distribution volumes in the steady state and terminal phase with low plasma clearance (Table 3). Maximum esomeprazole concentration was observed at 160±23.12 minutes after dosing. Cmax and CL values after oral administration were 35.23±7.69 and 1.31±0.13 respectively. The bioavailability of esomeprazole was estimated to be 61.33±19.45%, based on AUC $_{inf}$ ratios determined after oral value of immobilized and intravenous administration value of immobilized stress group.

3.5 Comparative pharmacokinetics

AUC_{inf}, Cmax, Vz, and CL values determined without immobilized stress were higher than those determined using the immobilized stress, presumably because immobilization stress interfered with drug absorption and gastric / hepatic blood flow [7,9]. In addition, the bioavailability of esomeprazole without immobolized stress was 85.33±21.23% and with immobolized stress was 61.33±19.45% which was 1.39 fold difference and significantly different ($p=0.0051$, $P<0.05$). The P values of differences between Cmax, T1/2, AUC_{inf}, CL, and Vss values determined using the two methods were 0.018, 0.016, 0.0037, 0.00651, and 0.00124, which represented significant differences ($p < 0.05$) indicates that immobilized stress play vitalrole in drug absorption.

In Conclusion, Stress, in any form, can alter pharmacokinetic parameters, and thus, it is important that stress be minimized to obtained reliable pharmacokinetic data. The present study demonstrates that the immobilized stress can significantly alters pharmacokinetics parameters.

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Table 1: Validation of the LC-MS/MS method for measuring esomeprazole in rabbit plasma

Parameters	Obtained Results
Lower limit of detection (µg/ml)	0.1 µg/ml
Calibration range (µg/ml)	0.1-100 µg/ml
Calibration equation	y = 0.42781x + 0.002214
Coefficient if regression(r^2)	0.99991
Interday Precision (CV %,n=5) [a]	
0.1 µg/ml	8.28
0.3 µg/ml	6.91
2 µg/ml	9.67
8 µg/ml	6.61
Interday Accuracy (%,n=5) [b]	
0.1 µg/ml	101.94
0.3 µg/ml	104.79
2 µg/ml	103.46
8 µg/ml	101.95
Intraday Precision (CV %,n=5) [a]	
0.1 µg/ml	13.31
0.3 µg/ml	5.16
2 µg/ml	3.25
8 µg/ml	3.68
Intraday Accuracy (%,n=5) [b]	
0.1 µg/ml	82.14
0.3 µg/ml	107.19
2 µg/ml	103.25
8 µg/ml	100.35
QC samples recovery (%,n=5)	
1 µg/ml	105.71
5 µg/ml	98.45
10 µg/ml	99.28

[a] %CV=Standard deviation of concentrations determined x 100/ Mean concentration determined

[b] Accuracy=Mean concentration determined x100/Concentration expected, The intra-and inter-day precisions expressed as coefficient of variations percent (% CV) should not exceed 15% at any concentration level, with the exception of LLOD, QC samples, where should not exceed ±20% (Bioanalytical Method Validation, FDA guidelines, May 2001).

Table 2:Non-compartmental pharmacokinetics of esomeprazole (40mg/kg) in rabbit plasma samples without immobilized stress

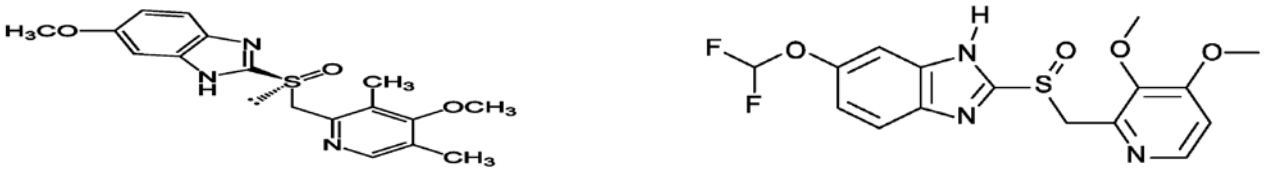
Parameters	Mean±SD
Oral Administration	
AUCin f(µg min /ml)	3125.63±218.77
Tmax(min)	120 ±14.27
Cmax (µg /ml)	57.24±3.08
T1/2(min)	404.22±22.00
CL(ml/min/kg)	1.97±0.14
Intravenous Administration	
AUCin f(µg min /ml)	3612.73±208.91
Cmax (µg /ml)	78.23±11.62
T1/2(min)	133.41±65.92
Vss(ml/kg)	1523.88±308.24
CL(ml/min/kg)	8.83±3.32
Bioavailability	**85.33±21.23%**

AUC:area under curve; Tmax: time to reach maximum concentration; Cmax: maximum concentration; T1/2: terminal half life; Vss : distribution volume in the steady state; CL: total clearance.

Table 3:Non-compartmental pharmacokinetics of esomeprazole (40mg/kg) in rabbit plasma samples acquired with immobilized stress

Parameters	Mean±SD
Oral Administration	
AUCinf(µg.min/ml)	2215.93±75.09
Tmax (min)	160 ±23.12
Cmax (µg /ml)	35.23±7.69
T1/2(min)	319.66±92.81
CL(ml/min/kg)	1.31±0.13
Bioavailability	**61.33±19.45%**

AUC: area under curve; Tmax: time to reach maximum concentration; Cmax: maximum concentration; T1/2: terminal half life; Vss,: distribution volume at the steady state; CL: total clearance



(A) **(B)**

Fig 1: Chemical Structure of esomeprazole (A) and Pantoprazole (B)

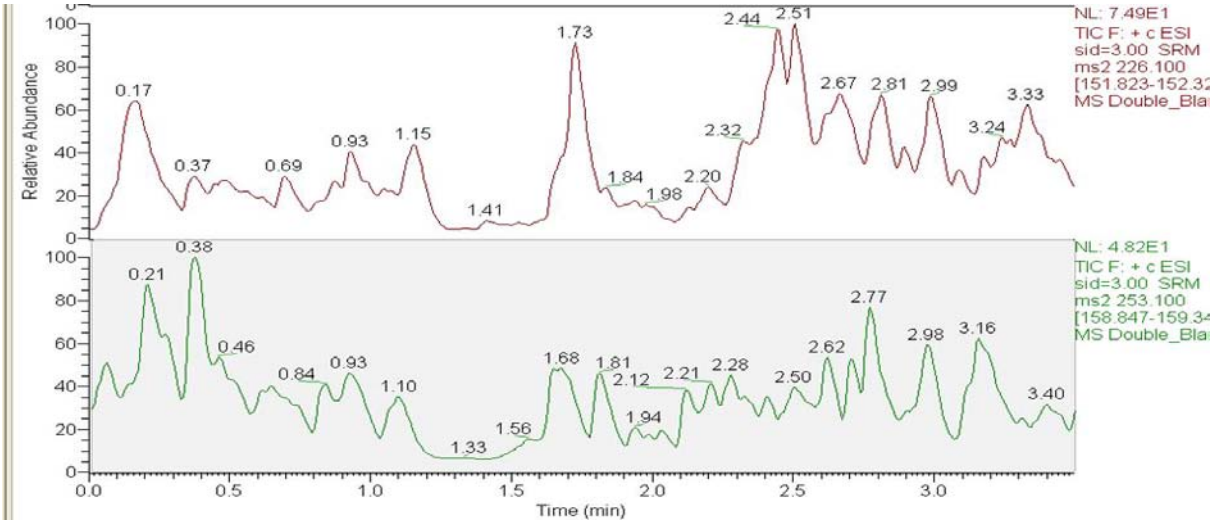


Fig 2a: No peaks corresponding to esomeprazole or the IS were observed in blank rabbit plasma using the LC-MS/MS conditions.

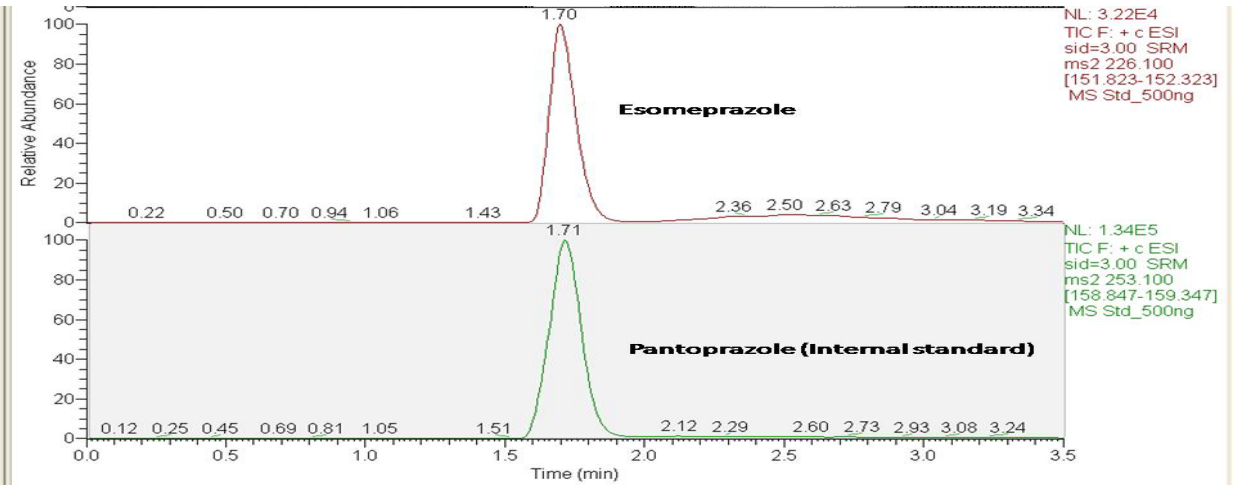


Fig 2b: A mass chromatogram of rabbit plasma spiked with esomeprazole and Pantoprazole (IS)

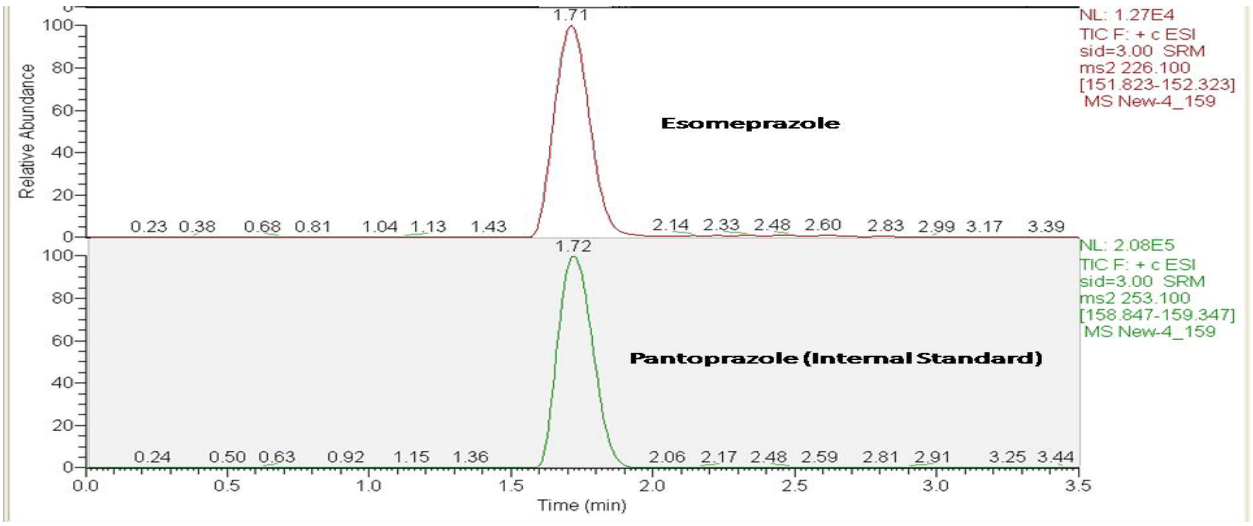


Fig 3: The mass chromatogram of blood samples at 120 min (oral study)

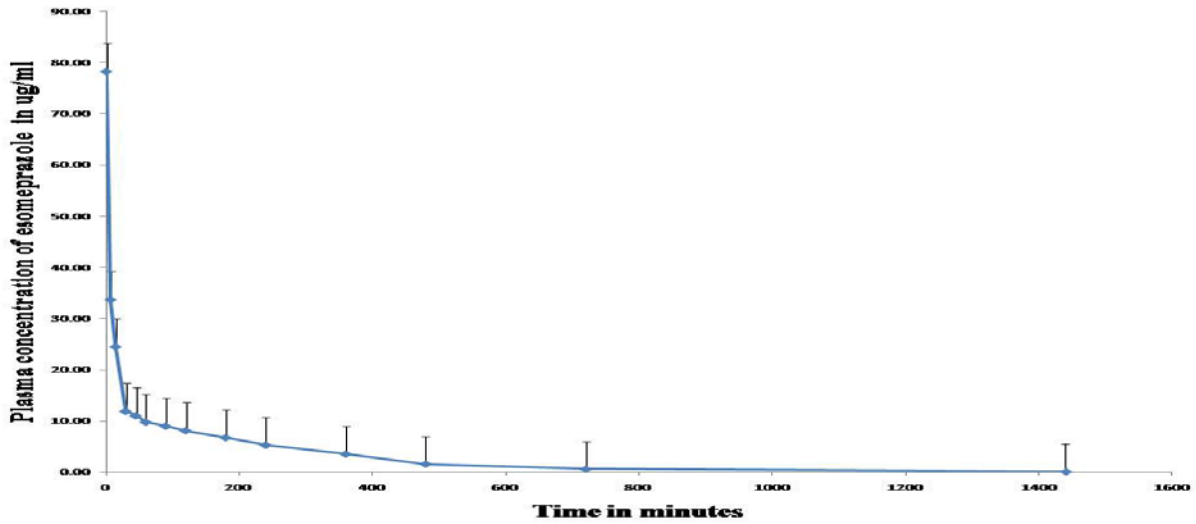


Fig 4b: The concentration–time profile of esomeprazole following its oral administration without immobilized stress

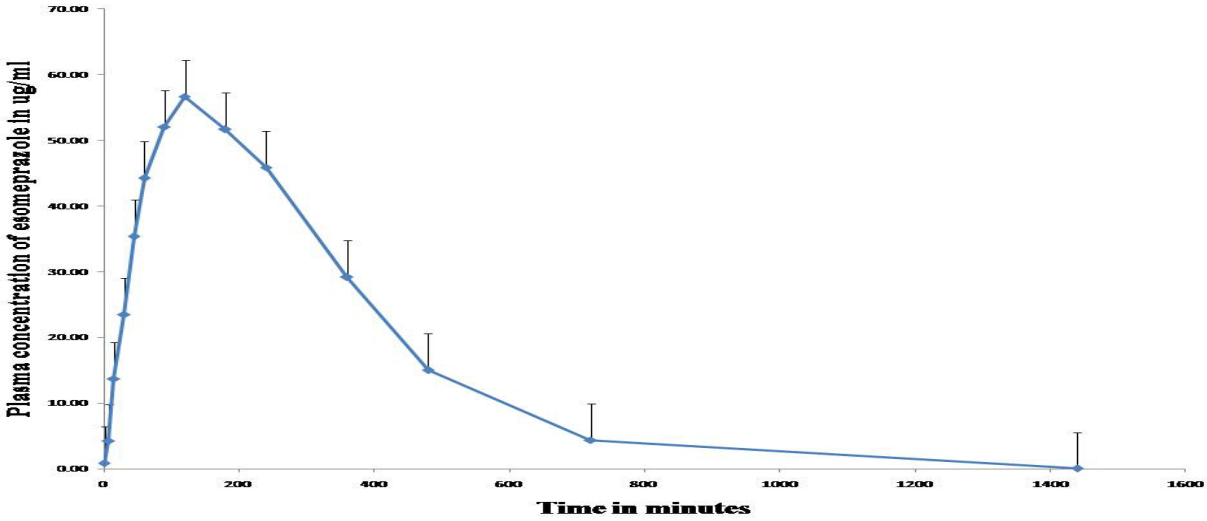


Fig 4b: The concentration–time profile of esomeprazole following its oral administration without immobilized stress

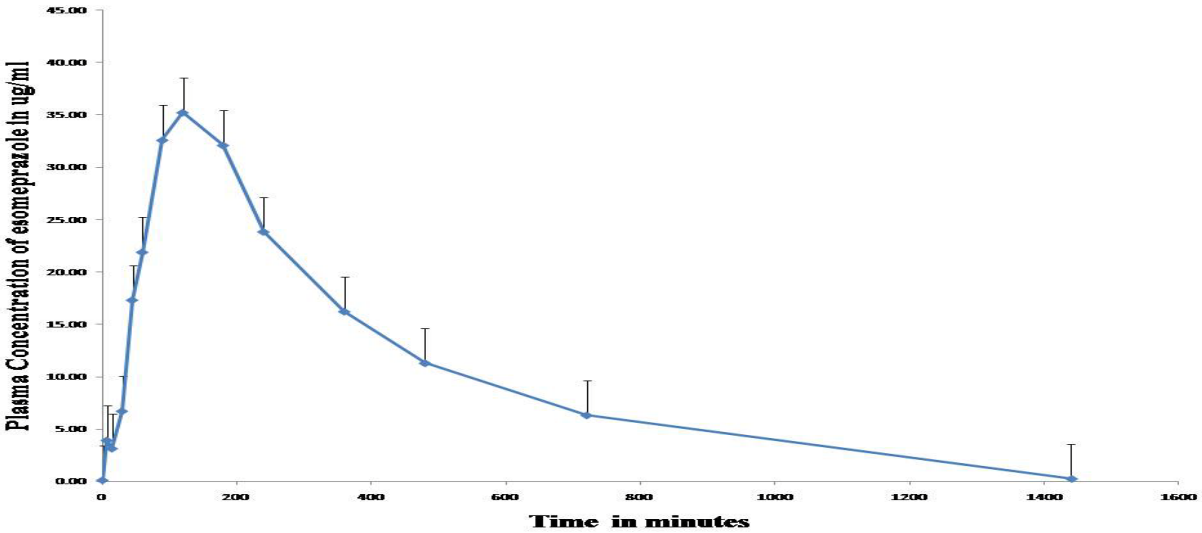


Fig 5: The concentration–time profile of esomeprazole following oral administration with immobilized stress

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