Renal clearance and urinary excretion of omeprazole in healthy female volunteers in Pakistan

Nazia Qadir[1], Tanweer Khaliq[1], Rai Altaf Hussain[2], Ghulam Abbas Shah[2], Sadia Asharaf[1], Ahmad Raza[1] and Nadia Noor[3]

[1]Department of Physiology and Pharmacology, University of agriculture, Faisalabad, Pakistan
[2]Department of Agronomy, PMAS- Arid Agriculture University Rawalpindi, Pakistan
[3]Department of Chemistry, University of agriculture, Faisalabad, Pakistan

Abstract: Omeprazole is a widely prescribed proton pump inhibitor to treat various gastric acid hyper secretion disorders. The present study was designed to evaluate the renal clearance and urinary excretion of omeprazole in eight healthy female volunteers to increase the understanding of the contributing factors such as demographics variability in the renal clearance and urinary excretion of omeprazole under indigenous conditions. The urine and blood samples were collected 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours after oral administration of enteric coated omeprazole (20 mg) and drug concentration in the samples was determined by High Performance Liquid Chromatography (HPLC) with C_{18} column and UV detector. Urinary excretion and renal clearance of omeprazole was calculated and data was statistically analyzed by using regression/correlation technique. Endogenous creatinine was also measured by reagent kit available in the market. The results indicate that mean diuresis was 0.0172±0.0029 ml/min/kg. While the mean values of renal clearance of creatinine and omeprazole were 1.315±0.103 and 0.066±0.0042 ml/min. kg, respectively. Whereas, clearance ratio was 0.055±0.007 which indicates back diffusion. The cumulative percentage of dose excreted was 6.71±0.358. A significant ($p<0.05$) negative correlation ($r= -0.457$) between clearance ratio and urine pH of omeprazole reflecting glomerular filtration reabsorption of drug at kidney tubular level while significant ($p<0.05$) negative correlation ($r= -0.681$) between clearance ratio and plasma concentration of omeprazole indicates the involvement of active tubular secretion of drug. It can be concluded that during glomerular filtration, omeprazole diffuse back/reabsorption. Therefore, Urinary excretion of omeprazole in indigenous healthy female subjects was observed to be lower than given in the literature values.

Keywords: Omeprazole, creatinine, clearance ratio, cumulative percentage of dose excreted.

INTRODUCTION

Omeprazole is proton pump inhibitor which is highly prescribed drug worldwide to treat various gastric acid hyper secretion disorders like peptic ulcer, gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome and heartburn (Kumar *et al.,* 2003; Hegar *et al.,* 2013). Primarily it is metabolized by hepatic cytochrome P450 isoenzyme CYP2C19 5-Hydroxy-Omeprazole (Ahmad *et al.,* 2011) but not metabolized significantly by CYP3A4 isoenzyme which is very important cytochrome isoenzyme responsible for metabolism of most drugs (Faruquee *et al.,* 2010). CYP2C19 enzyme is absent in about 3% of Caucasian and 15-20% Asian population. In these individuals Omeprazole is metabolized mainly by CYP3A4, after repeating dosing of drug the AUC is higher 10 times in people lacking CYP2C19 (Bertilsson *et al.,* 1997).

Renal clearance has significant effect on the pharmacology of drugs. Knowledge of renal clearance being most important part of total clearance that contributes in over dose management of drugs. Renal clearance plays specific part in knowing the drug interaction mechanisms of certain drugs. The study of urinary excretion of drugs provide important knowledge regarding distribution, absorption and excretion parameter of drug from body (Feng *et al.,* 2010). The inconsistency of urinary excretion of compounds in human beings is due environmental variation, plasma protein, drug metabolizing enzymes, urine pH renal blood flow, genetic differences, drug solubility and health state (Kurata *et al*., 2002).

Pakistan, like other developing countries, imports raw material and finished drugs for its human and veterinary health management. It has been reported that genetic makeup in local human, animals and environmental conditions are different than those of their foreign counterparts in drug manufacturing countries (Javed *et al.,* 2006). These differences suggested that disposition kinetic and bioavailability of different drugs should be investigated in species and the environment where drugs are going to be employed clinically.

On the basis of above mentioned facts the current project was planned to assess the renal clearance and urinary excretion of omeprazole in local population to increase the understanding of the contribution of factors such as

**Corresponding author:* e-mail: nazo_rai@yahoo.com

demographics variability in the renal clearance and urinary excretion of omeprazole in indigenous conditions.

MATERIALS AND METHODS

Protocol

The study was conducted in the Department of Physiology and Pharmacology, the University of Agriculture Faisalabad. A total of 8 healthy female subjects (Ten *et al.,* 1988) were chosen with age between 24-28 years, height between 162-170 cm and body weight between 53-64 kg. The subjects were asked to abstain from smoking, caffeinated beverages, chocolate and grape fruit prior and during the entire study as they interfere with cytochrome P450 enzymes which finally affect the drug metabolism. The subjects were given the same diet throughout the study period.

Study design

A single dose (20 mg omeprazole), non-controlled study was designed and performed in accordance with the principles of international conference on harmonization for conducting good clinical practice and declaration of Helsinki. The whole experiment was carried out in accordance with the guidelines of the directorate of graduate studies and institutional ethical committee of University of Agriculture, Faisalabad, Pakistan.

Ethical considerations

All subjects were informed about the study and experimental procedures completely in both verbal and written forms. Subjects were not allowed to any medication from 2 weeks before the study.

Drug

Cap omega® 20 mg and reference standard powder of omeprazole were used in the present study. After the overnight fasting, the selected female volunteers were given cap. Omega® 20 mg orally.

Collection of blood samples

Blood samples were collected at specific time intervals in heparinized plastic centrifuge tubes. Blank blood sample was collected before the administration of omeprazole as a control sample from each volunteer. Following drug administration at 8 am, the collection of blood samples was as 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours (Nazir *et al.,* 2013; Vlase *et al.,* 2010). The pH of each sample was measured by pH meter (Beckman HS, Germany) with glass electrode at 37°C. Blood samples were centrifuged at 4000 rpm for 30 minutes, plasma was separated and stored at -20°C.

Collection of urine samples

Before drug administration blank urine sample was collected from each volunteer before administration of drug. For renal clearance and urinary excretion the urine samples were collected at 45, 75, 105, 135, 165, 240, 360 and 480 minutes after administration of drug with water (250 ml) in plastic bottles containing 1 mol/L Na_2CO_3 to buffer the urine above pH 7 for preventing acidic degradation of drug. The volume of each collection of urine was measured. The pH of all urine samples was measured.

Plasma samples

To 1.0ml of plasma samples 500µl of 0.5M phosphate buffer (pH 8.0), 5ml of dichloromethane, 0.25g of sodium chloride and internal standard (phenacetin 100µl of a 0.1 mg/ml stock solution in menthol) were added test tubes. By using vortex miser samples were agitated for 10 mints and for about 30 mints at 4000 rpm were centrifuged, the aspirated superior aqueous layer was removed. In a new glass tube the remaining organic layer was shifted and at $40C^0$ was dried by using vacuum evaporator. By using mobile phase of 300µl the residue were reconstituted and filtered by passing through 0.45 45µm filter (Gelman Sciences, Ann Arbor, MI, USA). A 30µl volume of the filtrate was injected into the HPLC equipped with column C18 nova pack (75mm x 4.6mm, 3.5µm) and UV detector. Mobile phase was consisting of acetonitrile: Monopotassium phosphate solution 30mM in ratio of 33:67 (V/V) of pH 6.5 (Vlase *et al.,* 2010).

Urine samples

To 1.0ml of urine samples 500µl of 0.5M phosphate buffer (pH 8.0), 5ml of dichloromethane, 0.25g of sodium chloride and internal standard (phenacetin 100µl of a 0.2 mg/ ml stock solution in menthol) were added test tubes. By using vortex miser samples were agitated for 10 mints and for about 15 mints at 4000 rpm were centrifuged, the aspirated superior aqueous layer was removed. In a new glass tube the remaining organic layer was shifted and at $40C^0$ was dried by using vacuum evaporator. By using mobile phase of 200µl the residue were reconstituted and filtered by passing through 0.45µm filter. Injected volume of filtrate was 30 µl into HPLC apparatus.

Calibration curve

Calibration curve Stock solution of omeprazole was made up by dissolving 0.25 mg /ml of omeprazole in methanol and the make different dilutions in plasma and urine. After analysis the peak area versus concentrations were obtained to made standard cure of urine and plasma as shown in fig. 1 and 2.

Creatinine analysis

By using spectrophotometer (spectronic 212, Bausch & Lomb, Germany) following the method of Bonsnes and Taussky (1945) by Jaffe- reaction after treating creatinine reagent kit (Thomas, 1998) the concentration of creatinine in urine and plasma samples was calculated. The glomerular filtration rate (GFR) value was determined by using the renal clearance of endogenous creatinine.

Diuresis

The rate of urine flow in a time period was calculated as urine volume at specific collection time.

$$\text{Diuresis (mL/min/kg)} = \frac{\text{Volume of urine in a collection time period}}{\text{Time (min) x body weight (kg)}}$$

Renal clearance

It is defined as plasma volume being free of drug in given time per kg of body weight by kidneys. The glomerular filtration rate (GFR) was assessed by using the value of renal clearance of endogenous creatinine. Renal clearance endogenous creatinine and omeprazole was determined by the following formula by Swenson (1985):

$$CLren = \frac{Uc \times Uv}{Pc}$$

Where CL_{ren} is the renal clearance, Uc and Pc are concentration of a substance in urine and plasma respectively. Uv is urine rate flow (Diuresis, ml/min/kg).

Clearance ratio

It is calculated by dividing renal clearance of drug by creatinine renal clearance. Renal clearance of drug (Cld)

$$\text{Clearance Ratio} = \frac{\text{Renal clearance of drug (Cld)}}{\text{Renal clearance of creatinine (ClCr)}}$$

Effect of plasma drug concentration, rate of urine flow and urine pH was determined by regression correlation analysis.

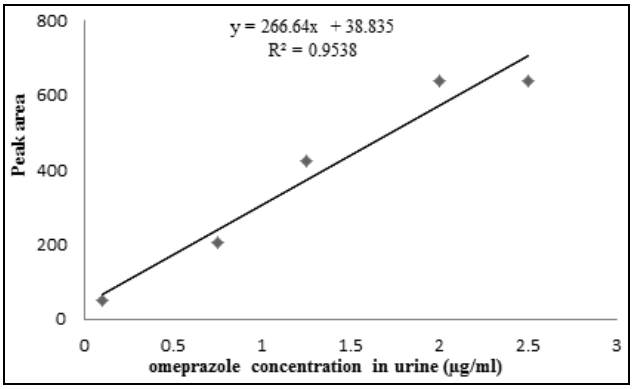

Fig. 1: Standard curve for analysis of omeprazole in urine

Urinary excretion

The mean ± SE values for the omeprazole at different time periods were determined in urine samples. Then cumulative percentage omeprazole dose excreted in the 8 hours after oral administration of omeprazole in urine was calculated by equations given below. Amount of omeprazole (mg) excreted in urine at different time intervals were calculated by using formula:

$$\text{Amount excreted in mg} = \frac{\text{Concentration of drug (µg/mL) x urine volume}}{1000}$$

Amount of omeprazole (mg) excreted at various time periods in urine samples was determined by using formula:

$$\text{Percent dose excreted} = \frac{\text{Amount excreted (mg)}}{\text{Amount of dose (mg)}} \times 100$$

Cumulative percent dose excreted in urine was calculated from the data of the Percent dose excreted of omeprazole in the urine of the female subjects.

STATISTICAL ANALYSIS

The mean (± SE) values for concentration of drug were calculated. Renal handling of omeprazole was assessed after its oral administration, effect of rate of urine flow (diuresis), plasma drug concentration and urine pH on its renal clearance was determined by using Microsoft Excel version 2003 via regression/correlation analysis.

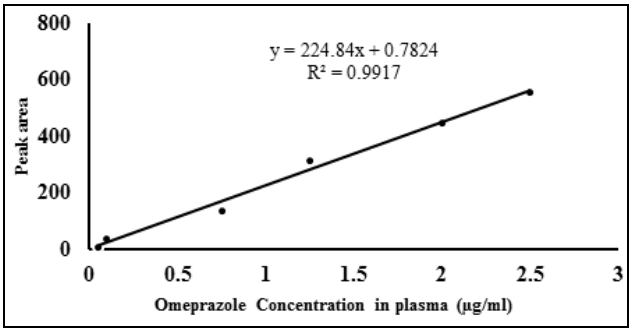

Fig. 2: Standard curve for analysis of omeprazole in plasma

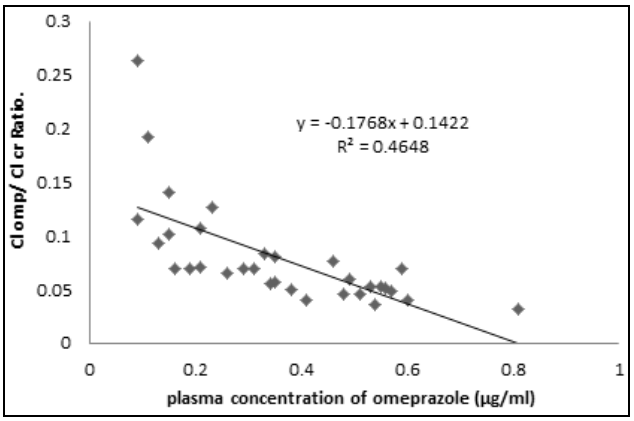

Fig. 3: Effect of plasma concentration of omeprazole on its renal clearance after oral dose of omeprazole 20mg in eight female subjects. Each data point shows one of the 32 observations in 8 experiments each comprised of 4 experimental periods.

RESULTS

Renal clearance

The values of diuresis, plasma, and urine concentration and renal clearance of endogenous creatinine and omeprazole are presented in the table 1. The values of rate of urine flow were 0.0712±0.003mL/min/kg. The pH in blood and urine was 7.43±0.007 and 6.13±0.21. The mean ±SE values for the concentration of endogenous creatinine in plasma and urine were 7.65±0.37 and 572.2 ±3.39µg/mL, while the respective values of omeprazole concentration was 0.306±0.001 and 1.22±0.28µg/mL. The

renal clearance of endogenous creatinine and omeprazole was 1.315±0.103 and 0.066±0.004mL/min/kg. The ratio between the clearance of omeprazole and the clearance of endogenous creatinine was 0.0556±0.007. The regression correlation analysis revealed a significant ($P<0.05$) negative correlation (r=-0.681) between plasma concentration of Omeprazole and its renal clearance (fig. 3). However, urine pH (r=-0.457) show negative correlation with the renal clearance of the drug (fig. 4).

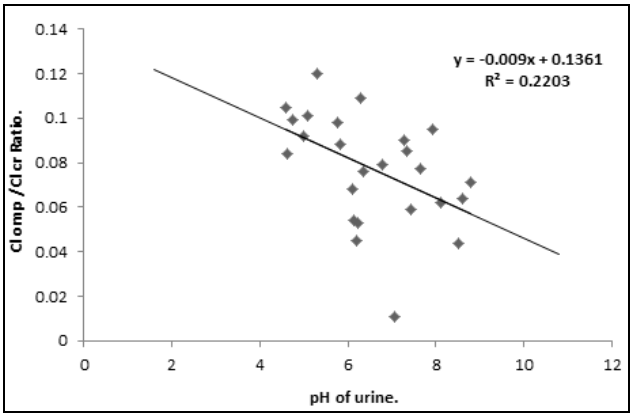

Fig. 4: Effect of urine pH of omeprazole on its renal clearance after oral dose of omeprazole 20mg in eight female subjects. Each data point shows one of the 32 observations in 8 experiments each comprised of 4 experimental periods.

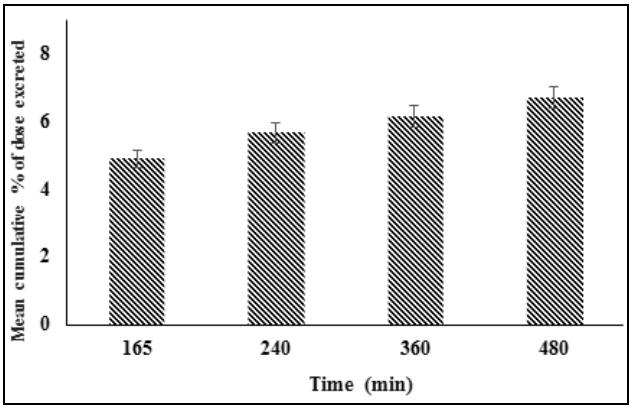

Fig. 5: Mean cumulative percent dose of omeprazole excreted in urine after oral dose of omeprazole 20mg in eight female subjects.

Urinary excretion

The mean ±SE values of the cumulative percentage of dose excreted presented in fig. 5. This fig. shows that the cumulative percentage of dose of omeprazole excreted at 8 h in the urine.

DISCUSSION

Omeprazole is an effective and suitable proton pump inhibitor which decreases acid production in the stomach. Bioavailability of omeprazole is significantly impairs by food, therefore, patients are advised to take OMP with an empty stomach with a glass of water. Plasma protein binding of omeprazole is approximately 95% (Regardh *et al.*, 1985). Half-life (T1/2) of omeprazole is less than one hr and it is cleared entirely from plasma within 3-4 hrs (Cederberg *et al.*, 1989). While omeprazole is mainly eliminated by kidneys nearly 77% in form of unchanged drug. Therefore study was planned to assess the renal clearance and urinary excretion of omeprazole in healthy female volunteer's under indigenous environment.

If the renal clearance of drug is higher than the creatinine clearance indicates that the drug undergoes tubular secretion. If the values of renal clearance of drug is less than the creatinine clearance it shows that drug is tightly bound to plasma protein or from site of renal tubules being under pass through the process of passive reabsorption. The elimination process waste substances should be done quickly from body as they are formed. The elimination of foreign and toxins substances are also done by kidney which either be ingested such as drugs, food additives and pesticides or formed by the body itself. Urine pH also has a great influence on whether a drug is excreted readily or slowly and in some clinical situations urine pH is maintained to control the excretion of certain drugs from the body.

Urine pH creates a vital part in the ionization of the drug and its absorption from the tubules. Most of the drugs are either weak acids or weak bases. Acidic drugs are more readily ionized in alkaline urine and alkaline drugs are more readily ionized in acidic urine. Ionized or polar substances are more soluble in water so readily dissolve in the body fluids for excretion. After oral administration of omeprazole 20mg to each subject the urinary excretion and renal clearance and of omeprazole were studied in eight healthy female subjects . The values of mean ±SE of diuresis was 0.017±0.0029 correlated with previous finding 0.018±0.0056 studied by (Waheed *et al.*, 2002). However present values of diuresis are higher than reported 0.012±0.002 previously in healthy males volunteers (Rao, 1997). The value of diuresis in present study was higher due to the reason that previous reported study was conducted in summer while current study was performed in winter. The difference is due to change of environment of winter and summer. As in summer evaporation via sweating causes reduced urine flow during summer, while lower temperature of winter increases the urine flow in winter (Nawaz and Shah, 1984). The pH of the urine of current study was 6.13±0.21 which is correlated to previously calculated pH of urine 6.13 0.185 (Ghani *et al.*, 2003) and 5.97±0.056 (Majeed *et al.*, 2003) and mean value of pH of urine of volunteer given omeprazole was 5.5±0.015 (Osther *et al.*, 1992). The pH of urine remains unaffected by omeprazole (Howden and Reid, 1984). Besides these seasonal changes pH of urine influenced by type of food intake in summer and winter season leading to lowering of pH of urine. The pH of the blood of current study was

7.43±0.004 which is correlated to previously studied pH of urine 7.43 0.021 (Rao, 1997) and 7.59±0.015 (Majeed *et al.,* 2003). The mean ±SE value of endogenous creatinine of plasma was 0. 76±0.037 mg/dl which is less than previously reported was 0.85±0.012 (Anquer *et al.,* 2010) and 0.92±0.021 (Lew and Bosch, 1991). Lower value of creatinine is difference of local species as previously reported studied were done in Western countries. The mean ±SE value of urinary creatinine was 57.2±0.329 mg/dl which within range (30- 300 mg/dl) as mentioned by (WHO, 1996) also correlated by previously finding 49.32±6.25 mg/dl reported (Majeed *et al.,* 2003). The mean ±SE value of renal clearance of endogenous creatinine of present study was 1.315±0.103 which is correlated by previously reported 1.43±0.3062 in female volunteers by (Majeed *et al.,* 2003) and 1.12±0.09 reported by (Rashid *et al.,* 2003) in male volunteers. The mean ±SE value of renal clearance ratio of omeprazole to creatinine of present study was 0.056±0.007. However the value of clearance ratio of drug to creatinine is less than 1 indicating reabsorption or back diffusion of drug (Hardman *et al.,* 1996). There is a significant ($P<0.05$) negative correlation ($r=-0.681$) between plasma concentration and clearance ratio of renal clearance of omeprazole and renal clearance of endogenous creatinine presented in. It reflects the saturation of excretory mechanism at higher plasma concentration of drug showing the occurrence of process of active tubular secretion. There is the negative correlation ($r= -0.457$) between pH of the urine clearance ratio of renal clearance of omeprazole and renal clearance of endogenous creatinine as $P<0.05$ showing a highly significant relationship between these two parameters presented in. As omeprazole is weak base by increasing the pH of the urine the acidic urine became alkaline the drug became unionized so reabsorbed and excretion decreases. As according to data basic drugs are more readily ionized in acidic urine and vice versa. Only unionized form of drug is reabsorbed while ionized or polar drugs are more soluble in water so readily dissolve in body fluids for excretion. It was concluded besides back diffusion or glomerular filtration reabsorption and active tubular secretion are also taking place in renal handling of omeprazole. The lower values of renal clearance support the previous findings that there is a need to evaluate the imported drug under indigenous conditions for getting optimal therapeutic effects and to highlight the lacunae in our knowledge of the drug's pharmacodynamics in human body.

Excretion of most of drugs is done via urine either as drug metabolite or as parent drug. Drugs are cleared via kidney by passing through same processes as kidney clearing endogenous substances which are included as passive tubular reabsorption, active tubular secretion and glomerular filtration. The quantity of drug being removed is the sum of the amount of drug being filtered and secreted minus amount of reabsorbed. The principle rout of the drug excretion is the urine. Our kidneys produce urine which contains urea, excess salts, drug metabolites and excess water. Kidneys perform two grand functions.

Table 1: Mean ±SE values of body weight, diuresis, plasma and urine concentration and renal clearance of endogenous creatinine and omeprazole in eight healthy female subjects after single oral dose of omeprazole 20 mg.

S. No	Body weight (kg)	Diuresis (ml/min/kg)	pH		Creatinine Conc. (µg/ml)		Renal Clearance of Creatinine (ml/min/kg) Cl_{cr}	Omeprazole Conc. (µg/ml)		Renal Clearance of Omeprazole Cl_{omp} (ml/min/kg)	Clearance Ratio. Cl_{omp}/Cl_{cr}
			Blood	Urine	Plasma	Urine		Plasma	Urine		
1	54	0.0188	7.43	6.63	8.2	494.0	1.36	0.292	1.22	0.091	0.067
2	55	0.0170	7.42	6.03	6.9	565.7	1.39	0.295	1.24	0.088	0.063
3	53	0.0160	7.45	6.9	7.3	461.7	1.01	0.351	1.09	0.040	0.039
4	64	0.0172	7.40	6.24	9.1	599.5	1.13	0.301	1.26	0.092	0.081
5	53	0.0171	7.46	5.39	6.9	621.5	1.54	0.280	1.20	0.081	0.052
6	55	0.0165	7.44	6.01	8.0	751	1.55	0.313	1.33	0.055	0.036
7	54	0.0163	7.43	5.45	9.0	474.2	0.85	0.292	1.28	0.070	0.082
8	53	0.0178	7.42	6.9	6.4	609.7	1.69	0.315	1.13	0.042	0.025
Mean ±SE	55.12 1.29	0.0172 0.0029	7.43 0.007	6.13 0.21	7.65 0.37	572.2 3.390	1.315 0.103	0.306 0.0078	1.22 0.028	0.066 0.0042	0.0556 0.007

First is to get rid of waste materials and second is to control the composition of the body fluids and the body volume. Renal excretion accounts for most drug elimination that are predominately ionized at physiological pH and for polar drugs, drug metabolites with low lipid solubility. Renal drug excretion decreases with aging. Drugs in unbound from are present in glomerular filtrate while drugs bounds to plasma proteins stay in circulation. Drugs and their metabolites only in Un-ionized states have a tendency to be reabsorbed from renal tubular fluids

Urinary excretion of endogenous and exogenous substances are manipulated by multiple mechanism of glomerular filtration, tubular and desorption in an active and passive way. Drugs are regarding as interfering with homeostasis and thus must be excreted by means of existing mechanism. The duration of drug action in the body is dependent on its elimination through metabolism and excretion. The cumulative percentage of dose excreted at 8 hours in urine of eight healthy female subjects of present study given in was 6.71±0.358 whereas the cumulative percentage of drug excreted in 48 hours was 8.23±3.32 investigated earlier (Faruqee *et al.*, 2010). Previously reported that hydroxyomeprazole excretion during the first 12hours varied between 4.6 to 15.5% of a given dose (Regårdh *et al.*, 1990). The cumulative percentage of dose excreted in 24 hours in rat after administration of 40 mg/kg oral omeprazole was 0.504±0.261, while it was 0.504±0.261 percent after intravenous omeprazole 20 mg/kg administration (Lee *et al.*, 2007).

CONCLUSIONS

Renal clearance of endogenous creatinine in indigenous healthy female subjects has been found lower than given in foreign counterparts. Moreover, glomerular filtration of omeprazole causes back diffusion/reabsorption and active tubular secretion. Therefore, Urinary excretion of omeprazole in indigenous healthy female subjects was observed to be lower than given in the literature values.

REFRENCES

Ahmad L, Iqbal Z, Nazir S, Shah Y, Khan A, Khan MI, Nasir F and Khan A (2011). Optimization and validation of HPLC-UV method for simultaneous determination of omeprazole and its metabolites in human plasma: Effects of various experimental conditions and parameters. *J. Liq. Chrom. Rel. Technol.*, **34**: 1488-1501.

Andersson T (1996). Pharmacokinetics, metabolism and interactions of acid pump inhibitors. Focus on omeprazole, lansoprazole and pantoprazole. *Clin. Pharmacok.*, **31**: 9-28.

Andersson T, Miners JO, Veronese ME and Birkett DJ (1994). Identification of human liver cytochrome P450 isoforms mediating secondary omeprazole metabolism. *Br. J. Clin. Pharmacol.*, **37**: 597-604.

Ansquer JC, Dalton RN, Causse E, Crimet D, Malicot KL and Foucher C (2008). Effect of fenofibrate on kidney function: a 6- week randomized crossover trial in healthy people. *Am. J. Kidney Dis.*, **51**: 904-913.

Bertilsson L, Tybring G, Widén J, Chang M and Tomson T (1997). Carbamazepine treatment induces the CYP3A4 catalysedsulphoxidation of omeprazole, but has no or less effect on hydroxylation via CYP2C19. *Br. J. Clin. Pharmacol.*, **44**: 186-189.

Bonsnes RM and Taussky HH (1945). On colorometric determination of creatinine by Jaffe reaction. *Biol. Chem.*, **158**: 581-591.

Cederberg C, Andersson T and Skanberg I (1989). Pharmacokinetics and metabolism in man. *Scand J. Gastroentro.*, **24**: 33-40.

Faruquee CF, Ashik U, Azad MAK, Rayhan I, Parveen S and Hasnat A (2010). Interaction between levofloxacin and omeprazole using urinary pharmacokinetic data. *Pak. J. Pharm Sci.*, **23**: 143-148.

Feng B, LaPerle JL, Varma MV and Chang G (2010). Renal clearance in drug discovery and development: Molecular descriptors, drug transporters and disease state. *Expert Opin. Drug Metab. Toxicol.*, **6**: 939-952.

Ghani F, Iqbal T and Shahid M (2003). Renal clearance of endogenous creatinine and doxycycline in male volunteers. *J. Med. Sci.*, **3**: 283-288.

Hardman JG, Limbird LE, Molinoff PB and Rudden RW (1996). Goodman and Gillman's the pharmacogical basis of therapeutics. 9thEd. The McGraw Hill companies, inc., New York. pp.199-242.

Hegar B, Hutapea EI, Advani N and Vandenplas Y (2013). A double-blind placebo-controlled randomized trial on probiotics in small bowel bacterial overgrowth in children treated with omeprazole. *J. pediat.*, **89**: 381-387.

Javed I, Nawaz M and Khan FH (2003). Pharmacokinetics and optimal dosage of kanamycin in domestic ruminant species. *Vet. Arhiv.*, **73**: 323-331.

Javed I, Rehman ZU, Khan FH, Muhammad F, Iqbal Z and Aslam B (2006). Renal clearance and urinary excretion of kanamycin in domestic ruminant species. *Pak. Vet. J.*, **26**: 1-8.

Kobayashi K, Chiba K, Sohn DR, Kato Y and Ishizaki T (1992). Simultaneous determination of omeprazole and its metabolites in plasma and urine by reversed-phase high-performance liquid chromatography with an alkaline-resistant polymer-coated C18 column. *J. Chrom. B.*, **579**: 299-305.

Kumar R, Chawla YK, Garg SK, Dixit RK, Satapathy SK, Dhiman RK and Bhargava VK (2003). Pharmacokinetics of omaprazole in patients with liver cirrhosis and extra hepatic portal venous obstruction. *Clin. Pharm.*, **25**: 625-630.

Kurata Y, Ieiri I, Kimura M, Morita T, Irie S, Urae A, Ohdo S, Ohtani H, Sawada Y, Higuchi S and Otsubo K (2002). Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin. Pharm Therap.,* **72**: 209-219.

Lee DY, Jung YS, Shin HS, Lee I, Kim YC and Lee MG (2007). Faster clearance of omeprazole in rats with acute renal failure induced by uranyl nitrate: Contribution of increased expression of hepatic cytochrome P450 (CYP) 3A1 and intestinal CYP1A and 3A subfamilies. *J. Pharm. Pharmacol.,* **60**: 843-857.

Lew SQ and Bosch JP (1991). Effect of diet on creatinine clearance and excretion in young and elderly healthy subjects and in patients with renal disease. *J. Am. Soc. Nephrol.,* **2**: 856-865.

Majeed B, Bhatti HN and Fatima K (2003). Renal Handling of in Female Volunteers. *Pak. J. Biol. Sci.,* **6**: 1191-1194.

Meyer UA (1996). Metabolic interactions of the proton-pump inhibitors lansoprazole, omeprazole and pantoprazole with other drugs. *Eur. J. Gastroenterol. Hepatol.*, **1**: 521-525.

Nawaz M (1994). Geonetical factors affecting bio disposition of drugs. *Can. J. Physiol. Pharmacol.,* **72**: 24-29.

Nawaz M and Shah BH (1984). Renal clearance of endogenous creatinine and urea in sheep during summer and winter. *Research in Veterinary Science,* **36**: 220-224.

Nazir S, Zafar I, Lateef A, Yasar S, Abad K, Fazli N and Muhammad A (2013). Pharmacokinetics of omeprazole and its metabolites in premenopausal and postmenopausal females. *Afr. J. Pharm. Pharmacol.,* **7**(16): 865-872.

Osther PJ, Rasmussen L and Pedersen SA (1992). A double-blind placebo-controlled trial of omeprazole on urinary pH in healthy subjects. *Urol. Neph.,* **24**: 229-231.

Rao AR (1997). Renal clearance and urinary excretion of ciprofloxacin in male volunteers. M.Sc. Thesis, Deptartment of Biochemistry, University of Agriculture Faisalabad, Pakistan.

Rashid S, Bhatti HN, Ijaz A, Sadaf B and Ahmed S (2003). Renal clearance of Acetylsalicylic acid in Human Male Volunteers. *Pak. J. Bio. Sci.,* **6**: 1399-1403.

Regardh CG, Andersson T, Lagerstrom PO, Lundborg P and Skanberg I (1990). The pharmacokinetics of omeprazole in humans-a study of single intravenous and oral doses. *Therap. Drug Mon.,* **12**: 163-72.

Ten Kate RW, Tuynman HA, Festen HP, Pals G and Meuwissen SG (1988). Effect of high dose omeprazole on gastric pepsin secretion and serum pepsinogen levels in man. *Eur. J. Clin. Pharmacol.,* **35**(2): 173-176.

Thomas L (1998). Clinical laboratory diagnostics. 1st Ed. TH-Books Verlagsgesellschaft, Frankfurt; pp.366-374.

Waheed N, Iqbal T, Sheikh MA and Khan FH (2002). Renal clearance of endogenous creatinine and levofloxacin in male volunteers. *J. Med. Sci.,* **2**: 145-148.

WHO (1996). Biological Monitoring of Chemical Exposure in the Workplace. Vol. 1. World Health Organization, Geneva, p.300.