Therapeutic Effect of Atorvastatin on kidney functions and urinary excretion of Glimepiride in healthy adult human male subjects

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Abstract: Glimepiride and Atorvastatin in combination are commonly employed for treating the hyperglycemia and dyslipidemia, respectively, in patients of type 2 diabetes. The present study was designed to find out the influence of Atorvastatin on urinary excretion and renal clearance of Glimepiride in healthy adult male volunteers. In each experimental subject, Glimepiride 2mg was given orally after an overnight fasting. Samples of blood and urine were taken at different specific time intervals. After a washout period of ten days, Glimepiride 2mg was co-administered with Atorvastatin 20mg orally. Post-medication, blood and urine samples were collected following the same sampling schedule as for Glimepiride alone. The samples were analyzed for Glimepiride and creatinine concentration by HPLC-UV and Spectrophotometer, respectively. Mean (±SE) values for blood pH 7.445±0.05 and 7.382±0.05, urine pH 4.972±0.08 and 5.08±0.10, diuresis 0.0207±0.00 and 0.0237±0.00ml/min/kg, endogenous creatinine in plasma 9.048±0.33 and 8.613±0.024µg/ml, endogenous creatinine in urine 512.34±18.20 and 556.72±4.60µg/ml, Glimepiride plasma concentration 0.16069±0.00 and 0.3227±0.01µg/ml, Glimepiride urine concentration 1.5994±0.03 and 0.8665±0.04µg/ml, renal clearance of creatinine 1.224±0.09 and 1.550±0.09ml/min/kg, renal clearance of Glimepiride 0.2064±0.01 and 0.0641±0.00ml/min/kg and clearance ratio 0.1791±0.01 and 0.0414±0.00 were observed for Glimepiride alone and its concurrent administration with Atorvastatin, respectively. Atorvastatin decreased the urinary excretion and renal clearance of Glimepiride due to which chances of hypoglycemia provokes and renal handling of Glimepiride involves back diffusion besides glomerular filtration and no influence of Atorvastatin was seen on these mechanisms.

Keywords: Drug interaction, Renal clearance, Healthy volunteers, Glimepiride, Atorvastatin.

INTRODUCTION

Diabetes mellitus is a growing epidemic disease in which impaired secretion of insulin or its decreased action at periphery results in chronic hyperglycemia which leads to induction of micro and macro vascular complications due to oxidative stress. Most of the vital organs e.g. nervous systems, eyes, heart, blood vessels and kidneys are affected due to long term complications of diabetes. Diabetes mellitus may also lead to development of insulin resistance, hypertension, obesity and dyslipidemia (Fowler, 2011). Diabetes is pharmacologically treated with oral hypoglycemic drugs along with lipid lowering, antihypertensive or antiplatelet drugs to minimize the cardiovascular complications and mortality (Gaede *et al*., 2008).

Sulphonylureas are widely used for the management of diabetes mellitus and Glimepiride, a blood glucose lowering agent, is an orally acting sulfonylurea. Glimepride has 100% systemic bioavailability and is metabolized by CYP2C9 (Chowdhury and Amajumdar, 2010; Aziz *et al.*, 2016). By the stimulation of insulin secretion from the pancreatic beta cells, it reduces the glucose level in blood and IR (insulin resistance) due to improvement in insulin sensitivity in peripheral tissues (Pawar *et al*., 2010). Atorvastatin is a lipid lowering drug that is specific, competitive and reversible inhibitor of the enzyme hydroxy methyl glutaryl coenzyme-A reductase (Nawroski *et al*., 1995) and can be employed therapeutically to treat hypercholesterolemia (Kwak *et al*., 2003). Statins have ability to moderately inhibit the metabolic enzymes such as CYP2C9, CYP2D6 and CYP3A4. So, there is probability of Atorvastatin for metabolic inhibition of glyburide, a member of sulfonylurea class which is metabolized by CYP2C9 and CYP3A4 (Aziz *et al.*, 2016). Statins along with anti-diabetic agents particularly with sulfonylureas are mostly used to correct dyslipidemia in diabetic patients. The alterations in pharmacokinetic parameters of

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sulfonylureas in co-administration with statin appear due to cytochrome P450 enzyme inhibition. Hence, it is purposeful to explore the influence of cytochrome enzyme inhibition on renal clearance and urinary excretion of Glimepiride, using Atorvastatin as CYP inhibitor. Moreover the concomitant use of sulfonylureas and statins is in practice clinically now-a-days. This research was designed to inquire the quantitative changes in renal clearance and urinary excretion of Glimepiride due to CYP2C9 inhibition by Atorvastatin.

MATERIALS AND METHODS

Experimental subjects

Ten healthy adult human male volunteers were selected from University of Agriculture, Faisalabad after ethical approval from relevant authority in the University. The volunteers were found to be in good health status and declared to be fit for this study by taking medical history, vital signs, general physical examination and blood and urine analysis. No medication was taken by any volunteer ten days before start of the study. Subjects having any kind of drug or food allergy were excluded from the study.

Drug

Glimepiride 2mg (Amaryl® 2mg tablets manufactured by Sanofi-Aventis Limited, Pakistan) and Atorvastatin 20mg (lipitor® 20mg tablets prepared by Pfizer Limited Karachi, Pakistan) were selected to administer in selected healthy subjects

Drug administration and collection of samples

Blood samples were drawn at 0.5, 01, 1.5, 02, 2.5, 3.5, 06, 08, 10 and 12 hours whereas urine samples were collected at 0.75, 1.25, 1.75, 2.25, 2.75, 04, 06, 08, 10 and 12 hour post administration of Glimepiride 2mg. Blank samples of blood and urine of each volunteer were also collected. After washout period of ten days, Glimepiride 2mg and Atorvastatin 20mg tablets were concomitantly administered in the same volunteers and blood and urine samples were collected following the same sampling schedule as that of Glimepiride alone. Plasma was separated after centrifugation of blood samples. The volumes of all the urine samples were noted and pH of each fresh sample of blood and urine was measured by pH meter. Both plasma and urine samples were preserved at temperature -20°C until assayed.

Analysis

Assay of Glimepiride

The quantity of Glimepiride in collected samples (blood and urine) was analyzed by HPLC method. Analysis was carried out using HPLC-sykam S1122 liquid chromatographic pump along with Sykam S3210 UV visible detector. The column Phenomenex Luna C18 (250×4.6mm, 5µm) was used. The 20µL of sample was injected through Sykam S5111 sample injector in each of the experiment. The isocratic mobile phase consisting of acetonitrile and 0.2M phosphate buffer of pH 7.4, 40:60 (v/v) was pumped at flow rate (1ml/min) through the column and the quantification of Glimepiride was achieved at wavelength 228nm. The mobile phase was filtered by using membrane filter (0.45µm) and then was sonicated before its use.

Determination of creatinine

The concentration of endogenous creatinine in samples of plasma and urine was determined by Jaffe-reaction using spectrophotometer (Perkin Elmer) according to the method of Bonsnes and Taussky (1945). Renal clearance of both the Glimepiride and creatinine was calculated. Renal clearance of the endogenous creatinine was used for the estimating the glomerular filtration rate (GFR).

Calculation

Diuresis

Rate of urine output is called diuresis

$$Diuresis = \frac{urine\ volume\ in\ collection period\ (ml)}{Time\ (min)\ X\ Body\ weight\ (kg)}$$

Renal clearance

It may be defined as the volume of plasma being cleared of drug by kidneys per unit time per Kg of body weight. Renal clearance of Glimepiride and of endogenous creatinine was determined by the following formula by (Waheed *et al.*, 2002).

$$Clren = Uc\ x\ Uv/Pc$$

Where,

- Clren is the renal clearance
- Pc and Uc are concentration of a drug in plasma and urine, respectively
- Uv is the urine flow rate (Diuresis, ml/min/Kg)

Clearance ratio

Ratio between renal clearance of drug and of creatinine was calculated by following formula:

$$Clearance\ ratio = \frac{renal\ clearance\ of\ drug}{renal\ clearance\ of\ creatinine}$$

Urinary excretion

Dose excreted in mg and percentage dose excreted of Glimepiride was calculated by following formulas:

$$Dose\ excreted\ in\ mg = \frac{Conc.of\ drug\ in\ \mu g\ Xurine\ vol.}{1000}$$

$$Percent\ dose\ excreted = \frac{Amount\ excreted\ in\ mg}{total\ dose}\ X\ 100$$

The dose excreted in mg versus time data was used to calculate the cumulative percent of the dose of Glimepiride excreted in the urine until 12 hours after medication.

STATISTICAL ANALYSIS

The mean value with standard error (mean±SE) was calculated for each concentration and parameter. The concentration of Glimepiride and endogenous creatinine in urine and plasma samples were used to calculate renal

clearance of Glimepiride and creatinine. Influence of plasma concentration of drug, diuresis and urine pH on renal clearance of the Glimepiride was determined by regression/correlation analysis. Urinary excretion of drug was expressed as cumulative percent of dose excreted (Steel *et al*., 1997). The figures and tables were prepared by Microsoft Excel version 2010.

RESULTS

Renal clearance

Comparison of mean ± SE values of Diuresis, pH of blood and urine, endogenous creatinine in samples (plasma and urine) and Glimepiride concentration in plasma and urine samples, renal clearance of creatinine and of Glimepiride in adult human male volunteers following single oral administration of Glimepiride 2mg and its concurrent oral administration with Atorvastatin 20mg has been shown in table 1. The creatinine clearance was measured as an index of GFR (glomerular filtration rate).

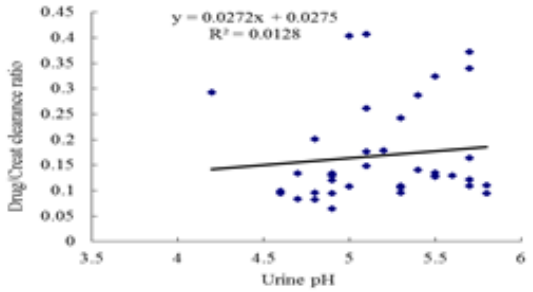

Fig. 1: Effect of urine pH on renal clearance of Glimepiride 2mg after oral administration in healthy male subjects.

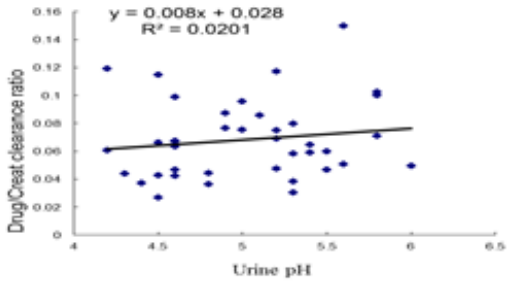

Fig. 2: Effect of urine pH on renal clearance of Glimepiride 2mg in combination with Atorvastatin 20mg after oral administration in healthy male subjects.

The mean±SE values of renal clearance for creatinine in healthy adult males following single oral administration of Glimepiride 2mg was 1.224±0.09ml/min/kg which significantly (P℟0.05) increased to 1.550±0.09ml/min/kg followed by concomitant administration of Glimepiride 2mg with Atorvastatin 20mg. The renal clearance of Glimepiride after single and concomitant oral administration with Atorvastatin was 0.2064±0.01 and 0.0641±0.00ml/min/kg, respectively, which represented a significant (P℟0.05) decreasing trend.

The effect of urinary pH and diuresis on ratio between renal clearance of Glimepiride and creatinine after giving Glimepiride 2mg alone and along with Atorvastatin 20mg demonstrated a non-significant (P 0.05) positive correlation (fig. 1-fig. 4) while non-significant (P 0.05) negative correlation was observed between the plasma concentration of Glimepiride and renal clearance ratio of Glimepiride and endogenous creatinine after alone (fig. 5) and concurrent (fig. 6) administration in ten healthy adult human male subjects. Each data point shows one of the 40 observations in 10 experiments each comprised of 4 experimental periods**.** The renal clearance ratio between Glimepiride and creatinine was less than 1 after giving dose of Glimepiride alone and along with Atorvastatin which indicated back diffusion along with glomerular filtration. Clearance ratio of Glimepiride alone was significantly (P℟0.05) higher than that of its concomitant administration with Atorvastatin. Renal clearance of Glimepiride alone was significantly (P 0.05) greater than that of the combined form with Atorvastatin (table 1).

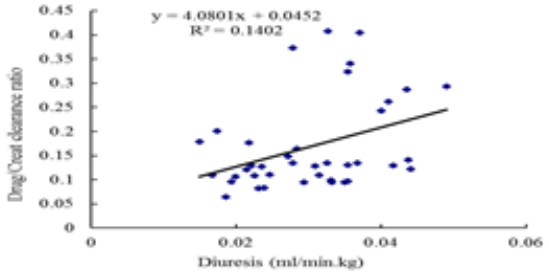

Fig. 3: Effect of diuresis on renal clearance of Glimepiride 2mg after single oral administration in healthy male subjects.

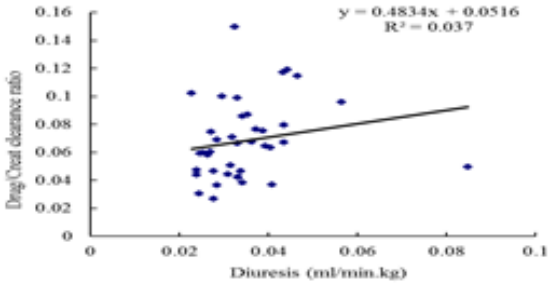

Fig. 4: Effect of diuresis on renal clearance of Glimepiride 2mg in combination with Atorvastatin 20mg after oral administration in healthy male subjects.

Urinary excretion

Mean ± SE values for cumulative percent of Glimepiride excreted via urine up to 12hours after single oral administration in healthy male subjects was 56.094±1.86% (fig. 7) and after its concomitant administration with Atorvastatin was 37.089±3.03% (fig. 8). The results in table 2 show that dose excreted in mg

and cumulative percent of excreted dose following administration of Glimepiride 2mg alone was reduced significantly (Pℜ0.05) upon its concurrent administration with Atorvastatin 20mg. The decreased urinary excretion of Glimepiride upon concurrent administration with Atorvastatin was also demonstrated by reduced values of renal clearance in the same combination.

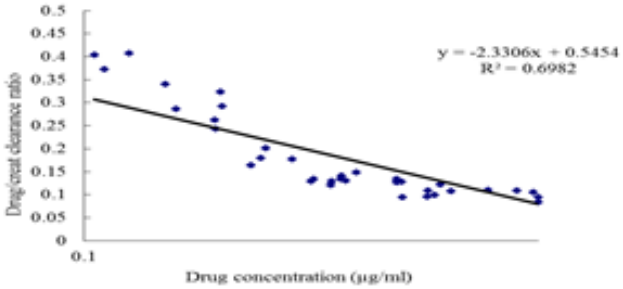

Fig. 5: Effect of plasma concentration of drug on the renal clearance of Glimepiride 2mg after single oral administration in healthy male subjects.

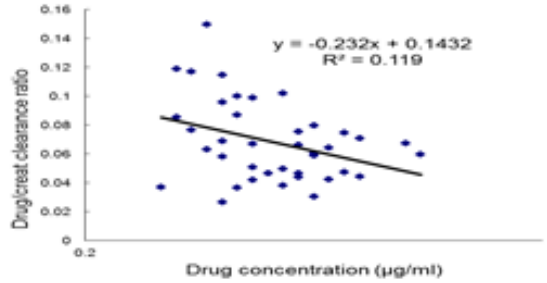

Fig. 6: Effect of plasma drug concentration on renal clearance of Glimepiride 2mg in combination with Atorvastatin 20mg after oral administration in healthy male subjects.

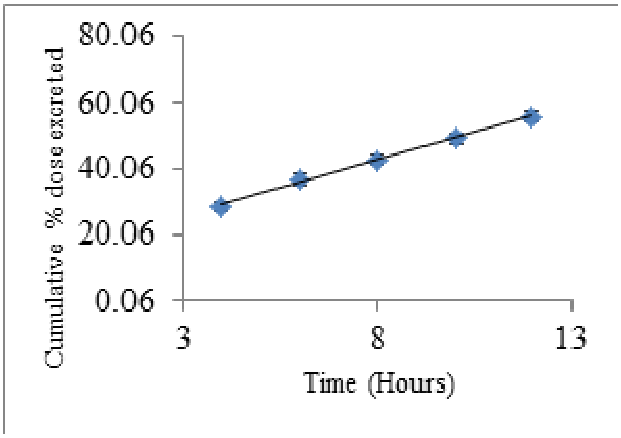

Fig. 7: Mean ± SE cumulative percent of dose excreted in urine after single oral dose administration of Glimepiride 2mg in 10 healthy male subjects.

DISCUSSION

The mammalian system has highly developed anatomical arrangement in which renal cells are interposed between blood and urinary passages. Kidney maintains the homeostasis of body fluids. Measurement of the kidney function is very important for diagnosis and the management of the renal diseases (Zitta *et al*., 2002). The metabolic waste products are excreted through kidneys including urea, uric acid, creatinine, end product of hemoglobin breakdown (bilirubin) and hormone metabolites. The rapid elimination of waste products is as necessary as they produced in the body. Nephron, the basic unit of kidney, performs three different functions namely glomerular filtration, reabsorption and tubular secretion. Due to glomerular filtration relatively smaller molecules will appear in the urine. This filtration requires a sufficient blood pressure to allow the filtrate and to counter balance the pressure exerted by plasma proteins. The passive filtration as well as active secretion may occur due to various proteins which are specific for acidic and basic compounds (Ullrich, 1994). In tubular reabsorption exchange of molecules takes place from the lumen (of nephron) into blood. Both the active and passive mechanisms are involved here. Due to re-absorption equilibrium is established again between unbound drug in plasma and urine. The elimination process is modified by the change in pH of the urine. The acidic drugs are excreted easily and rapidly in basic urine than that of the basic compounds (Anwar *et al.*, 2015).

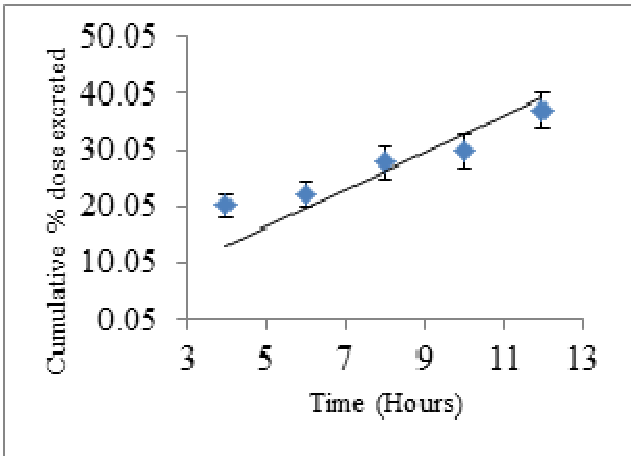

Fig. 8: Mean ± SE cumulative percent of dose excreted via urine after single oral dose administration of Glimepiride 2mg following concurrent oral administration of Atorvastatin 20mg orally in 10 healthy male subjects.

To develop tools for the prediction of drug clearance, a lot of efforts have been made (Naritomi *et al*., 2001). The clearance of creatinine will be equal to glomerular filtration rate (GFR). In case of net tubular secretion the clearance of drug exceeds GFR and tubular re-absorption will occur if the clearance is less than GFR (Ganong, 2005). Different physiological and biochemical factors such as pH, enzymes, dieresis etc. may affect the renal clearance of a drug, like Glimepiride (Anwar *et al*., 2015).

The renal clearance can be defined as the volume of the plasma being cleared of drug/substance per unit time via

Table 1: Renal clearance of Glimepiride 2mg in healthy male subjects after its administration as single oral dose and in combination with atorvastatin 20mg.

S. No.	Parameters		Glimepiride alone	Glimepiride with atorvastatin
1	Diuresis (ml/min.kg)		0.0207±0.00	0.0237±0.00
2	pH	Blood	7.445±0.05	7.382±0.05
		Urine	4.972±0.08	5.08±0.10
3	Creatinine concentration (µg/ml)	Plasma	9.048±0.33	8.613±0.024
		Urine	512.34±18.20	556.72±4.60
4	Glimepiride concentration (µg/ml)	Plasma	0.1606±0.00	0.3227±0.01*
		Urine	1.5994±0.03	0.8665±0.04*
5	Renal clearance (ml/min/kg)	Creatinine	1.224±0.09	1.550±0.09
		Glimepiride	0.2064±0.01	0.0641±0.00*
6	Clearance ratio	Glimepiride/Creatinine	0.1791±0.01	0.0414±0.00*

Each value represents the mean of four observations in four experimental periods in ten volunteers * = Significantly (P 0.05) different from respective values

Table 2: Comparison of mean ± SE values of Glimepiride excreted in the urine of human male volunteers following its 2mg alone and concomitant oral administration with Atorvastatin 20mg.

Parameters	Glimepiride alone	Glimepiride with Atorvastatin
Cumulative dose excreted in mg up to 12 hr	1.116±0.007	0.0737±0.009*
Cumulative % dose excreted	56.094±1.86	37.089±3.03*

* = Significantly (Pℜ0.05) different from respective values

kidney. The amount of drug which is primarily filtered by glomerular filtration with negligible tubular re-absorption and tubular secretion will have the renal clearance almost equal to the creatinine clearance that normally is about 100ml/min in adults. The drug clearance greater than the creatinine clearance shows that the drug undergoes tubular secretion. Whereas, the drugl clearance lesser than the creatinine renal clearance is indicative of high plasma protein binding of drug and passive re-absorption from renal tubules (Anwar *et al*., 2015).

Environmental and genetic differences play a greater role in the disposition kinetics of a drug. These differences are also manifested through the variation in urinary excretion and renal clearance of a drug suggesting that disposition kinetic, bioavailability, urinary excretion and renal clearance of this drug should be investigated in species and the environment where the drug is clinically going to be employed (Ashraf *et al*., 2015; Aziz *et al.,* 2016).

Renal clearance of Glimepiride in adult males was calculated as 0.2064±0.01ml/min/kg which significantly (P 0.05) decreased upon its concomitant administration with Atorvastatin to 0.0641±0.00ml/min/kg. The clearance ratio of Glimepiride also decreased significantly (Pℜ0.05) from 0.1791±0.01 on single dose administration to 0.0414±0.00 on combination with Atorvastatin. Lesser than unity value of clearance ratio of Glimepiride in both the cases showed re-absorption or back diffusion of the drug (Anwar *et al*., 2015). As in the present study the clearance ratio is less than one in both experimental phases so it suggests that passive re-absorption is involved in renal handling of Glimepiride after single and concomitant administration with Atorvastatin besides glomerular filtration.

The renal excretion of Glimepiride which may be either slow or rapid depends upon urine pH that imparts its vital role in ionization of Glimepiride (drug). Mostly, drugs are weak acids or bases. When urine is alkaline in nature, the ionization of acidic drug like Glimepiride is more and vice versa. Consequently, its solubility being ionized moiety was increased in bodily fluids, hence suitable for excretion. In current study, relationship between clearance ratio and urine pH was noted to be non-significant (P℘0.05) positive correlation in both phases having r-value 0.086 (Glimepiride alone) and r-value 0.149 (in combination with Atorvastatin). This depicted that renal excretion of Glimepiride is directly proportional to urine pH (fig. 1-2).

Similarly the influence of diuresis on ratio between clearance of Glimepiride and creatinine after giving Glimepiride alone and along with Atorvastatin represented a non-significant (P℘0.05) positive correlation having correlation (r) values 0.382 and 0.187, respectively. This showed that upon lower diuresis the clearance of Glimepiride was less and residence time of drug in body was more. Consequently, it was assumed that in renal handling of Glimepiride passive re-absorption was involved rather than glomerular filtration (fig. 3-4).

As per the relationship between clearance ratio and plasma drug concentration was concerned, a non-

significant ($P \wp 0.05$) negative correlation was noted in both phases having correlation (r) values, -0.837 when Glimepiride alone was given and -0.345 when Glimepiride was given with Atorvastatin. This demonstrated that at higher values of plasma drug concentration, the excretory mechanisms would be saturated revealing that active tubular secretion was also involved (fig. 5-6).

The urinary excretions in terms of the cumulative percent dose of Glimepiride excreted via urine upto 12 hours after administration of Glimepiride alone and its co-administration with Atorvastatin have been shown in fig. 8 and fig. 9, respectively.

The mean value of cumulative percent dose excreted of Glimepiride alone was 56.084±1.86% and following its co-administration with Atorvastatin was 37.089±3.03%. Both values are variable significantly ($P \Re 0.05$) representing about 33.8% reduction (table 2).

In present study, lower urinary excretion of Glimepiride alone as compared to its co-administration with Atorvastatin also shown by respective results of its renal handling. The results demonstrate that rather than glomerular filtration, the dose administered was also absorbed through back diffusion at tubular level. Furthermore, following co-administration of Glimepiride with Atorvastatin GFR is found lower and subsequently urinary excretion of Glimepiride will be less. Moreover, pH of urine remained non-significantly ($P \wp 0.05$) variable but reduced after co-administration of Glimepiride with Atorvastatin (table 1). At comparatively lower level of urinary pH, the ionization of a weak acidic drug such as Glimepiride reduces giving way to unionization that is suitable for its absorption at level of kidney tubules.

CONCLUSION

The Atorvastatin significantly ($P \Re 0.05$) reduced the urinary excretion and renal clearance of Glimepiride which resulted in raised concentration of drug in plasma, hence, may lead to hypoglycemia. As this combination is worthwhile, so, dose adjustment should be considered. The renal handling of Glimepiride involved back diffusion besides glomerular filtration. However, no influence of Atorvastatin was observed on these mechanisms in healthy adult human male subjects.

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