Quercetin does not alter the oral bioavailability of Atorvastatin in rats

Rekha Koritala[1], Siva Reddy Challa[2*], Satheesh Kumar Ragam[1], Lal Babu Geddam[1], Venkatesh Reddy Challa[1], Renuka Devi [1], Srinu Sattenapalli[1] and Narendra Babu[3]

[1]Department of Pharmacology, AMR Memorial College of Pharmacy, Narasarao Pet, Guntur, India
[2]Department of Pharmacology, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, India
[3]Director, Cystron Pharmaceutical Laboratories, Autonagar, Vijayawada, India

Abstract: The study was undertaken to evaluate the effect of Quercetin on the pharmacokinetics of Atorvastatin Calcium. *In-vivo* Pharmacokinetic studies were performed on rats in a single dose study and multiple dose study. Rats were treated with Quercetin (10mg/kg) and Atorvastatin Calcium (20mg/kg) orally and blood samples were collected at (0) pretreatment and 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, 12, 24 hours post treatment. Plasma concentrations of Atorvastatin were estimated by HPLC method. Quercetin treatment did not significantly alter the pharmacokinetic parameters of atorvastatin like AUC_{0-24}, $AUC_{0-\alpha}$, T_{max}, C_{max} and $T_{1/2}$ in both single dose and multiple dose studies of Atorvastatin Calcium. Quercetin does not alter the oral bioavailability of Atorvastatin Calcium in rats.

Keywords: Atorvastatin calcium, bioavailability, CYP3A4, P-glycoprotein, pharmacokinetics, quercetin.

INTRODUCTION

Atorvastatin Calcium is an established cardio protective drug in myocardial infarction (Teshima *et al*., 2009); Atar *et al*., 2006) and Quercetin is also known to generate cardioprotective actions supported by experimental studies (Annapurna *et al*., 2009; Punithavathi & Stanely Mainzen Prince, 2011). The literature reveals that Atorvastatin Calcium is a P-glycoprotein (P-gp) inhibitor (Yang *et al*., 2011) and CYP3A4 substrate even as Quercetin is a well-known P-gp and CYP3A4 inhibitor.

Due to the ability of flavonoids to inhibit both p-gp and CYP3A4, flavonoids have been gaining lots of attention and are being exploited as bioenhancers to get enhanced oral drug bioavailability of poor bioavailable drugs whose poor bioavailability is mainly recognized to either p-gp mechanism or CYP3A4 mechanism (Fasinu *et al*., 2013). Considering the poor bioavailability of Atorvastatin Calcium and modulation of p-gp by these two cardio protective drugs (Atorvastatin Calcium and Quercetin), it is quite logical and rational to study the pharmacokinetics of Atorvastatin Calcium administered orally following pre-treatment of Quercetin. Hence, this study was undertaken to evaluate the effect of Quercetin on the pharmacokinetics of Atorvastatin Calcium.

MATERIALS AND METHODS

Drugs and chemicals

Atorvastatin Calcium was kindly offered from Matrix Laboratories, Hyderabad (India). Quercetin was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. All HPLC grade solvents (acetonitrile, methanol and water) were procured from SD Fine chemicals, Mumbai, India. All other chemicals used were of analytical grade and purchased from local chemical agencies.

**Corresponding author:* e-mail: sivareddypharma@gmail.com

Equipments

Waters HPLC system equipped with Waters 717 Autosampler, Waters 486 UV Detector and Waters 600 pump with Empower-2 Software was used. Column used was Zodiac C8, 150mm x 4.6mm, 5µm.

Animals

Albino Wistar rats (National Institute of Nutrition, Hyderabad, India), of either sex, weighing 200-250g, were selected. Animals were maintained under standard laboratory conditions at 25±2ºC, relative humidity 50±15% and normal photoperiod (12h dark/12h light). Commercial pellet diet (Rayon's Biotechnology Pvt Ltd, India) and water were provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee of AMR Memorial College of Pharmacy on 04-05-2012 with protocol no: AMRMCP/IAEC/2012/13 and experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Institutional CPCSEA registration number is CPCSEA/ORG/CH/2008/Reg.no.1219.

Pharmacokinetic study in rats

Experimental procedure

Wistar rats were randomly distributed into four groups of six animals in each group. Before doing, all experimental animals were fasted for 18h and but water was given *ad libitum.* After collection of initial blood samples, drugs were administered in the following order.

Group I Control (0.2ml of 0.9% *carboxy methyl cellulose (*CMC) sodium; p.o.)

Group II Atorvastatin Calcium (20 mg/kg; p.o.)
Group III (Single dose study) Atorvastatin Calcium (20 mg/kg; p.o.) + (Quercetin (10 mg/kg; p.o.)
Group IV (Multiple dose study) Atorvastatin Calcium (20 mg/kg; p.o.) + (Quercetin (10 mg/kg; p.o.)

In a single dose study, pretreatment sample was collected and then, quercetin suspension was administered 15 min before administration of Atorvastatin Calcium suspension followed by blood sample collection at various time points. In a multiple dose study, quercetin and Atorvastatin Calcium were daily administered for 7 days and on the 8th day, following administration of drugs and blood samples were collected at various time points.

Preparation of drug suspension
About 20mg of Atorvastatin was accurately weighed before being triturated in a dry clean mortar with an addition of 30µL of tween 80 and then, required volume of 0.9% sodium CMC was added and triturated again to suspend the drug in it. Then, suspension was transferred to plastic vials. Drugs were administered to the animals within 10 minutes of the preparation of the suspension**.**

Blood sample collection from rats
In this study, blood samples were collected at time points, pretreatment (0), 0.5, 1,1.5, 2, 2.5, 3, 4, 8, 12, 24 hours post treatment from the retro-orbital sinuses using fine capillary tubes into 2 ml Eppendorf tubes containing sodium citrate as an anticoagulant. Plasma was separated by centrifugation at 5000RPM/10 min and stored at -20°C until further analysis. Plasma concentration of Atorvastatin was estimated by HPLC method.

Estimation of Atorvastatin by HPLC method
Chromatographic conditions
The mobile phase consisted of a buffer (About 3.40 grams of Potassium dihydrogen orthophosphate taken into 1litre water and p^{H}-2.0 was adjusted with orthophosphoric acid) and acetonitrile in the ratio of (50: 50). The injection volume was 20µL. The mobile phase was delivered at 1.0 ml/min. The mobile phase was filtered through 0.22µm membrane filter. The flow rate was adjusted to 1.5ml/min and the effluent was monitored at 205nm. The total run time of the method was set at 11min. Retention time was obtained at 5-6 mins.

Preparation for calibration curve of Atorvastatin for in-vivo samples
Preparation of Linearity solution
Linearity solutions of various concentrations were prepared ranging from 0.2 microgram to 1.5 microgram per ml of Atorvastatin Calcium in plasma.

Sample preparation
To about 100µL of sample, about 100µl of the mobile phase buffer was added and was mixed well. Further, about 400µL of acetonitrile was added to precipitate all the proteins and mixed in vortex cyclomixture. Then, these were centrifuged at 4000 RPM for 15-20 min and supernatant solution was collected in HPLC vial and was injected into HPLC and chromatogram was recorded.

Construction of calibration curve
Linearity solutions were injected and noted the response of each solution. A linear relationship was obtained between the peak area and the corresponding concentrations. The slope of the plot was used to calculate the Atorvastatin concentration in the unknown sample. A linear calibration curve in the range of 200 µg-1500µg per ml was established (r^2=0.999).

STATISTICAL ANALYSIS

Results were expressed as mean ± S.D. Comparisons of plasma concentration vs. time profiles of Atorvastatin Calcium alone group and Atorvastatin Calcium with the quercetin combination group were analyzed using two way ANOVA followed by Bonferroni post hoc test whereas comparisons of pharmacokinetic parameters of these two groups were analyzed using unpaired student 't' test.

RESULTS

Calibration curve
Linear relationship was obtained between the peak area and the corresponding concentrations (fig. 2). The equations of linear regression were performed using the least-square method. Retention time was obtained at 5-6 min. Chromatogram was shown in fig. 1.

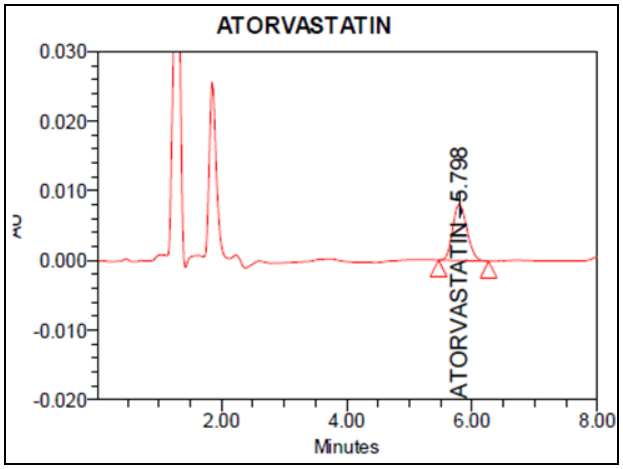

Fig. 1: Chromatogram of Atorvastatin at 2µg/ml

Effect of Quercetin on plasma concentration time profiles of Atorvastatin Calcium in a single dose study
The plasma concentration vs time profiles of Atorvastatin in rats following oral treatment of Atorvastatin Calcium with and without Quercetin were shown in table 1 and fig.

3. From the comparison of plasma concentration profiles of Atorvastatin Calcium in the absence and presence of Quercetin, it is clear that there is no significant elevation of plasma concentration of Atorvastatin Calcium in the combination group except at 0.5hr ($p<0.001$) post treatment (T_{max} of combination group) when compared to 0.5hours of Atorvastatin alone group. Line graph (fig. 3) clearly shows that the Atorvastatin Calcium concentrations in the combination group as well as Atorvastatin Calcium alone groups were present at 24th hour. These clearly indicate the increased elimination half-life of the drug and mean retention time of the drug in the body.

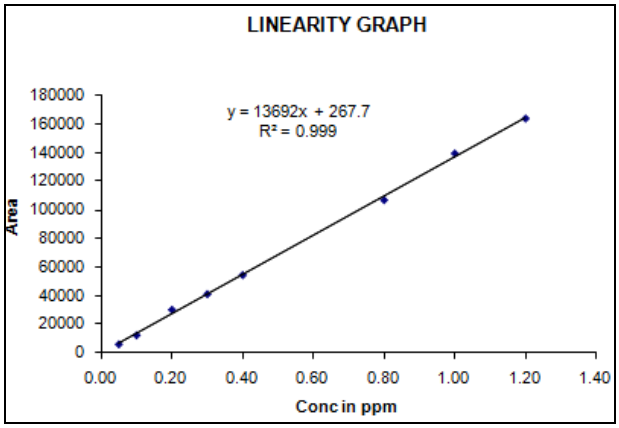

Fig. 2: Calibration curve of Atorvastatin plotted between concentration (µg/mL) and peak area (m^2)

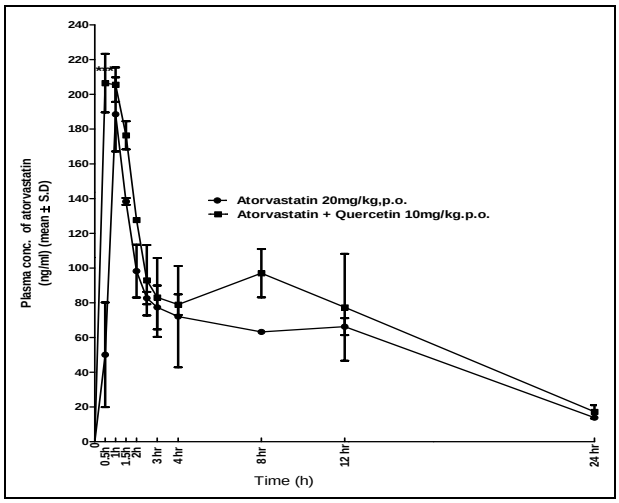

Fig. 3: Plasma concentration time profiles of Atorvastatin in the absence and presence of Quercetin in a single dose study

N=5; All values are represented as Mean ± S.D; ***p<0.001Vs Atorvastatin group analyzed by Two way ANOVA followed by Bonferroni post-hoc test.

Effect of Quercetin on plasma concentration time profiles of Atorvastatin Calcium in multiple dose study

The plasma concentration V's time profiles of Atorvastatin Calcium in rats following oral treatment of Atorvastatin Calcium with and without Quercetin were shown in table 2 and fig. 4. From the comparison of plasma concentration profiles of Atorvastatin Calcium in the absence and presence of Quercetin, it is clear that there is no significant elevation of plasma concentration of Atorvastatin Calcium in the combination group except at 0.5 hr ($p<0.001$) post treatment (T_{max} of combination group) when compared to 0.5hours of Atorvastatin Calcium alone group. Line graph (fig. 4) clearly speaks that the Atorvastatin concentrations in the combination group as well as Atorvastatin Calcium alone groups were present at 24th hour. These clearly indicate the improved elimination half-life of the drug and mean retention time of the drug in the body.

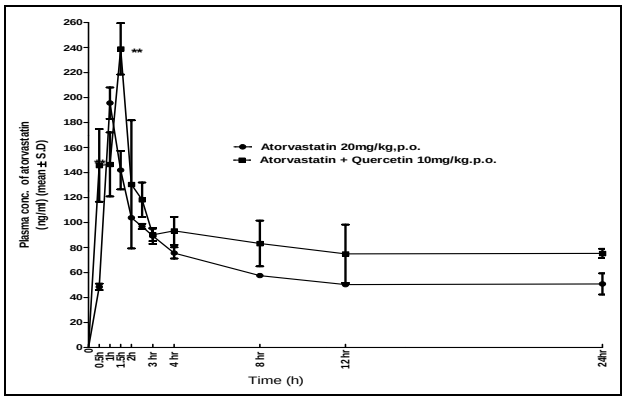

Fig. 4: Plasma concentration time profiles of Atorvastatin in the absence and presence of Quercetin in a multiple dose study

N=5; All values are represented as Mean ± S.D; ***p<0.001Vs Atorvastatin group analyzed by Two way ANOVA followed by Bonferroni post-hoc test

Effect of quercetin on pharmacokinetic parameters of atorvastatin calcium

The pharmacokinetic parameters of Atorvastatin Calcium were calculated using Try-Kinetica software and the parameters include half-life ($t_{1/2}$), clearance (CL), volume of distribution (Vd), maximum concentration (C_{max}), time to reach maximum concentration (Tmax) and area under the curve (AUC). The calculated pharmacokinetic parameters of Atorvastatin Calcium in rats for both single dose study and multiple dose study were shown in table 2 and table 3 respectively.

DISCUSSION

The results of this pharmacokinetic (PK) study reveal that Quercetin (10mg/kg, p.o.) increases the bioavailability of Atorvastatin Calcium (20mg/kg, p.o.) in a single dose acute study which was clearly evident from the significant elevation of C_{max} ($p<0.01$), AUC_{0-24} ($p<0.001$), AUC_{0-inf} ($p<0.01$) and $T_{1/2}$ ($p<0.05$) of Atorvastatin Calcium in the combination group when compared to the PK parameters of Atorvastatin Calcium alone group was observed.

Table 1: Plasma concentration time profiles of Atorvastatin in the absence and presence of Quercetin in a single dose study

	Atorvastatin 20mg/kg	Atorvastatin 20mg + Quercetin 10mg
Time points	Mean ±S.D	Mean ±S.D
0	0.0±0.0	0.0±0.0
0.5	50.06±52.17	206.51±29.16***
1	188.50±36.98	205.58±17.10^{NS}
1.5	138.37±3.33	176.46±14.05***
2	98.28±26.38	127.73±12.0 ^{NS}
2.5	82.69±6.12	92.97±35.16 ^{NS}
3	77.37±21.85	83.1±39.42 ^{NS}
4	72.04±50.53	78.95±10.24 ^{NS}
8	63.27±1.442	97.07±24.14 ^{NS}
12	66.23±8.47	77.42±53.20 ^{NS}
24	13.90±1.71	17.24±6.54 ^{NS}

Table 2: Plasma concentration time profiles of Atorvastatin in the absence and presence of Quercetin in a single dose study

	Atorvastatin 20mg/kg	Atorvastatin 20mg + Quercetin10mg
Time points	Mean ±S.D	Mean ± S.D
0	0±0	0±0
0.5	48.50± 4.51	145.69±50.29**
1	195.565±21.73	146.52±44.35 ^{NS}
1.5	141.925±26.83	238.96±35.62**
2	103.77±0.32	130.54±88.74 ^{NS}
2.5	96.87±3.63	118.26±23.84 ^{NS}
3	89.21±11.10	90.16±8.66 ^{NS}
4	75.63±7.79	93.27±19.43 ^{NS}
8	57.57±2.91	83.25±31.79 ^{NS}
12	50.31±0.25	74.93±40.52 ^{NS}
24	50.85±14.61	75.27±6.52 ^{NS}

The data is represented as mean ± S.D; n = 5; Statistical analysis was performed by comparing to Atorvastatin group using unpaired student 't' test: **p<0.05 vs Atorvastatin group with respective time point; NS: Not significant

Table 3: Comparison of pharmacokinetic parameters of Atorvastatin in rats treated with Atorvastatin alone and Atorvastatin in combination with Quercetin in a single dose study

	Atorvastatin	Atorvastatin and Quercetin
C_{max} (ng/ml)	168.835±9.17	207.76±77.40 ^{NS}
T_{max}(hr)	1.0±0.0	0.75±0.35 ^{NS}
AUC_{0-24} (ng.hr/ml)	1289.00±247.43	1657.05±510.47 ^{NS}
$AUC_{0-\infty}$(ng.hr/ml)	1432.62±294.55	1847.61±361.25 ^{NS}
$t_{1/2}$ (h)	7.06±1.13	7.02774±3.331972 ^{NS}
MRT_{0-24} (hr)	9.02±0.35	8.45±0.12 ^{NS}
$MRT_{0-\infty}$ (hr)	11.52±0.16	11.61±3.07 ^{NS}
Clearance (L/h)	0.0142±0.002	0.01103±0.002 ^{NS}
Volume of distribution (V_d) (L/hr)	0.076±0.087	0.1170±0.0749 ^{NS}

The data is represented as mean ± S.D; n = 5; Statistical analysis was performed by comparing to Atorvastatin group using unpaired student 't' test: NS: Not significant

Our hypothesis that the simultaneous ingestion of the flavonol, quercetin might increase the bioavailability of Atorvastatin Calcium by inhibiting CYP3A4 activity is clearly not supported by the present results. It is a rationale to presume the possible drug interaction between atorvastatin (CYP3A4 substrate) and Quercetin (CYP3A4 inhibitor).

Table 4: Comparison of pharmacokinetic parameters of Atorvastatin in rats treated with Atorvastatin alone and Atorvastatin in combination with Quercetin in a multiple dose study

	Atorvastatin	Atorvastatin and Quercetin
C_{max}(ng/ml)	195.56±91.735	313.44±171.98 ^{NS}
T_{max}(hr)	1.0±0.0	1.33±0.76 ^{NS}
AUC_{0-24} (ng/ml/h)	1482.27±246.06	2007.87±517.77 ^{NS}
$AUC_{0-\infty}$(ng/ml/h)	3528.13±334.06	5190.26±1413.74 ^{NS}
$t_{1/2}$ (h)	28.30±2.95	28.78±13.96 ^{NS}
MRT_{0-24} (hr)	10.51±1.49	11.11±0.62 ^{NS}
$MRT_{0-\infty}$ (hr)	41.72±1.02	44.56±20.90 ^{NS}
Clearance (L/h)	0.0056±0.0005	0.0040±0.0009 ^{NS}
Volume of distribution (V_d) (L/hr)	0.2337±0.046	0.1543±0.0317 ^{NS}

The data is represented as mean ± S.D; n=5; Statistical analysis was performed by comparing to Atorvastatin group using unpaired student 't' test: NS: Not significant

The synergistic barrier function of CYP3A4 and P-gp in the small bowel played a vital role in the biological defense mechanism to xenobiotics. Based on *in-vitro* confirmation, quercetin is an inhibitor of CYP3A4 and P-gp, and thus it is likely to increase the oral bioavailability of Atorvastatin Calcium. Results of *in vivo* single dose and multiple dose studies indicated that quercetin has not significantly altered the Atorvastatin Calcium oral bioavailability, thus, one can exclude an inhibitory effect of quercetin on CYP3A4-mediated metabolism of Atorvastatin Calcium, although quercetin did inhibit CYP3A4 in human liver microsomes (Ha *et al*., 1995; Miniscalco *et al*., 1992; Schubert *et al*., 1995). Although we neither decisive the expression or activity of CYP3A4 nor tissue concentrations of quercetin in this study, a very likely clarification for the lack of CYP3A4 inhibition by the quercetin *in vivo* could be the fact that hepatic quercetin concentrations did not get to a level that was shown to be effective in *in-vitro* experiments. In addition to its diminished tissue concentrations, quercetin itself undergoes an extensive first pass metabolism representing conjugated metabolites with diverse pharmacological properties from the parent compound quercetin (Cermak & Wolffram, 2006; Kroon *et al*., 2004). From this standpoint, it is speculated that the effect of quercetin on the providence of Atorvastatin Calcium cannot be attributed to its modulation of CYP3A4. However, few studies reported that quercetin induces CYP3A4 enzyme which was reflected by quercetin induced accumulation of CYP3A4 mRNA in culture human hepatocytes (Raucy, 2003). Another study indicated improved expression of CYP3A4 mRNA in Caco-2 cells following exposure of herbal agents (hyperforin, kaempferol and quercetin) (Pal & Mitra, 2006). If quercetin increases CYP3A4 induction, it should decrease the plasma concentrations of Atorvastatin Calcium, but quercetin neither decrease nor increase the plasma concentrations of atorvastatin Calcium in the present study.

Earlier results have revealed that Grapefruit juice increases serum concentrations of Atorvastatin Calcium (Lilja *et al*., 1999; Fukazawa *et al*., 2004). This effect is probably not accredited to quercetin because the key constituents of grapefruit juice are naringenin, naringein and hesperedin etc.

The organic anion transporting polypeptide 1B1 (OATP1B1) plays significant role in the hepatic uptake of the rather hydrophilic atorvastatin. The fibrate, gemfibrozil was exposed to raise plasma levels of Atorvastatin (Whitfield *et al*., 2011). Since quercetin or its plasma metabolites increased Atorvastatin Calcium concentrations, we can eliminate an effect of the quercetin on hepatic OATP1B1 under the present conditions. This surveillance is in accordance with an *in- vitro* study in which quercetin also failed to change the activity of this transporter (Wang *et al*., 2005).

CONCLUSION

In summary, Quercetin does not alter the oral bioavailability of Atorvastatin Calcium in rat models in both single dose study and multiple dose study as well. Lack of interaction between these two drugs could be of clinical significance. However, further studies are needed to confirm the absence of interaction between these two drugs in humans.

REFERENCES

Annapurna A, Reddy CS, Akondi RB and Rao SR (2009). Cardioprotective actions of two bioflavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats. *J. Pharm. Pharmacol.,* **61**(10): 1365-1374.

Atar S, Ye Y, Lin Y, Freeberg SY, Nishi SP, Rosanio S, Huang MH, Uretsky BF, Perez-Polo JR and Birnbaum Y (2006). Atorvastatin-induced cardio-protection is mediated by increasing inducible nitric oxide synthase and consequent S-nitrosylation of cyclooxygenase-2. *Am. J. Physiol. Heart Circ. Physiol*., **290**(5): 1960-1968.

Cermak R and Wolffram S (2006). The potential of flavonoids to influence drug metabolism and pharmacokinetics by local gastrointestinal mechanisms. *Curr Drug Metab.,* **7**: 729-744.

Fasinu PS, Gutmann H, Schiller H, James AD, Bouic PJ and Rosenkranz B (2013). The potential of Sutherlandia frutescens for herb-drug interaction. *Drug Metab Dispos.,* **41**(2): 488-497.

Fukazawa I, Uchida N, Uchida E and Yasuhara H (2004). Effects of grapefruit juice on pharmacokinetics of atorvastatin and pravastatin in Japanese. *Br. J. Clin. Pharmacol*., **57**(4): 448-455.

Ha HR, Chen J, Leuenberger PM, Freiburghaus AU and Follath F (1995). *In vitro* inhibition of midazolam and quinidine metabolism by flavonoids. *Eur. J. Clin. Pharmacol.,* **48**: 367-371.

Kroon PA, Clifford MN, Crozier A, Day AJ, Donovan JL, Manach C and Williamson G (2004). How should we assess the effects of exposure to dietary polyphenols *in vitro*. *Am. J. Clin. Nutr.,* **80**: 15-21.

Lilja JJ, Kivisto KT and Neuvonen PJ (1999). Grapefruit juice increases serum concentrations of atorvastatin and has no effect on pravastatin. *Clin. Pharmacol. Ther*., **66**(2): 118-127.

Miniscalco A, Lundahl J, Regardh CG, Edgar B and Eriksson UG (1992). Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. *J. Pharmacol. Exp. Ther.,* **261**: 1195-1199.

Pal D and Mitra AK (2006). MDR- and CYP3A4-mediated drug-herbal interactions. *Life Sci*., **78**(18): 2131-2145.

Punithavathi VR and Stanely Mainzen Prince P (2011). The cardioprotective effects of a combination of quercetin and α-tocopherol on isoproterenol-induced myocardial infarcted rats. *J. Biochem. Mol. Toxicol.,* **25**(1): 28-40.

Raucy JL (2003). Regulation of CYP3A4 expression in human hepatocytes by pharmaceuticals and natural products. *Drug Metab. Dispos.,* **31**: 533-539.

Schubert W, Eriksson U, Edgar B, Cullberg G and Hedner T (1995). Flavonoids in grapefruit juice inhibit the *in vitro* hepatic metabolism of 17b-estradiol. *Eur. J. Drug Metab. Pharmacokinet.,* **20**: 219-224.

Teshima Y, Yufu K, Akioka H, Iwao T, Anan F, Nakagawa M, Yonemochi H, Takahashi N, Hara M and Saikawa T (2009). Early atorvastatin therapy improves cardiac function in patients with acute myocardial infarction. *J. Cardiol.,* **53**(1): 58-64.

Wang X, Wolkoff AW and Morris ME (2005). Flavonoids as a novel class of human organic anion-transporting polypeptide OATP1B1 (OATP-C) modulators. *Drug Metab. Dispos.,* **33**: 1666-1672.

Whitfield LR, Porcari AR, Alvey C, Abel R, Bullen W and Hartman D (2011). Effect of gemfibrozil and fenofibrate on the pharmacokinetics of atorvastatin. *J. Clin. Pharmacol.,* **51**(3): 378-388.

Yang SH, Choi JS and Choi DH (2011). Effects of HMG-CoA reductase inhibitors on the pharmacokinetics of losartan and its main metabolite EXP-3174 in rats: Possible role of CYP3A4 and P-gp inhibition by HMG-CoA reductase inhibitors. *Pharmacology*, **88**(1-2): 1-9.