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Pharmacokinetic Analysis of Atorvastatin with Silymarin and Rutin in Hepatotoxic Rats with a Special Reference to Functional Status of CYP3A4 Enzyme

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ABSTRACT

The study was conducted to assess the functional status of CYP3A4 substrate (atorvastatin) in hepatotoxicity model treated with silymarin and rutin for a period of 14 days in rats. Hepatotoxicity was induced with acetaminophen (500 mg/Kg *po* once daily for 3 days) in adult male Wistar rats in 3 groups. Group 1: Normal control, Group 2, 3 and 4 were administered distilled water (5 ml/kg *po* once daily), silymarin (25 mg/Kg *po* once daily) and rutin (20 mg/Kg *po* once daily), respectively subsequently for 11 days from the last dose of acetaminophen. On the 15th day, a CYP3A4 substrate (atorvastatin @ 10 mg/kg *po*) was administered in all the groups and blood samples were collected at predetermined intervals. Pharmacokinetic interaction studies were conducted for evaluation of CYP3A4 activity using the specific substrate atorvastatin in all the groups. Mean plasma concentration (C_{max}), half-life ($t_{1/2}\beta$), area under the plasma concentration time curve (AUC) and mean residence time (MRT) of groups 2 and 4 were significantly ($p<0.05$) increased and elimination rate constant (β) was significantly ($p<0.05$) decreased in acetaminophen treated group as comparison to the normal control group. The kinetic profile of silymarin-treated group 3 was comparable to group 1 for single dose study. All the pharmacokinetic parameters of atorvastatin revealed significant correlations between hepatotoxic control and rutin treated group, while silymarin treated group showed significant alterations in the kinetic profile suggesting its hepatoprotective effect.

Keywords: Acetaminophen, Atorvastatin, CYP3A4, Rutin, Silymarin

Drug induced hepatotoxicity mimics both acute and chronic forms of liver disease (Kaplowitz, 2004). Drug induced hepatotoxicity accounts for the withdrawal of substantial amounts of clinically approved drugs from the market (Kim *et al*., 2016). Most of the drugs are metabolized in liver affects the organ's functional activity causing drug-induced injury (Kaplowitz, 1996).

Acetaminophen (N-acetyl-p-aminophenol/APAP; PCM) acts as antipyretic/analgesic agents were commonly used in the treatment of inflammatory conditions (Hinson *et al*., 2010; Aycan *et al*., 2014). The microsomal enzyme bio-activates acetaminophen to a reactive metabolite (N-acetyl-para benzoquinone imine/NAPQI) in the liver. NAPQI binds with cellular proteins covalently, deplete hepatic glutathione, and substantially cause centrilobular hepatic necrosis in a dose-dependent fashion (Mitchell *et al*., 1973; Zhang *et al*., 2013). Acetaminophen overdose is the most frequent cause of drug-induced acute liver failure and used as a reliable model for assessing the hepatoprotective potential of drugs (Olaleye *et al*., 2014).

Atorvastatin is reversible HMG-CoA reductase inhibitor that are primarily metabolized by cytochrome P450 (CYP3A4) contributes to the reduction of cardiovascular disease worldwide (Shitara and Sugiyama, 2006; Vladutiu, 2008). The co-administration of statins with drugs that inhibit CPY3A4 isoenzyme potentially increase the risk of adverse effects as the metabolism of statins gets slowed down (Rowan, 2010).

Many natural products of herbal origin are in use for the treatment of liver ailments (Mitra *et al*., 2000). Rutin (rutoside, quercetin-3-O-rutinoside and sophorin) is a natural glycoside between the flavonol quercetin and the

disaccharide rutinose with strong anti-oxidant property. The anti-inflammatory properties of rutin implied increased liver antioxidant enzymes in CCl_4-induced liver injuries in rats (Khan *et al*., 2012) and also countered the expression levels of inflammatory markers induced by a high-cholesterol diet (Sikder *et al*., 2014) by preventing oxidative stress, inflammation and cell death.

Silymarin, a flavonolignan isolated from the seeds and fruits of *Silybum marianum*, commonly known as 'milk thistle' (Vargas-Mendoza *et al*., 2014). Silymarin provides hepatoprotective effects against various toxic models of experimental liver diseases in laboratory animals (Muriel and Mourelle, 1990; Muriel *et al*., 1992; Carducci *et al*., 1996; Fraschini *et al*., 2002).

Hence, the study was conducted to investigate the metabolic function of CYP3A4 in acetaminophen-induced hepatic damage and the ameliorative effects of silymarin and rutin by assessing the pharmacokinetics of the specific substrate atorvastatin among rats in hepatotoxicity.

MATERIALS AND METHODS

Acetaminophen and rutin were procured from Himedia, India and silymarin from SRL, India and all the above drugs were administered in distilled water. Chemicals used in the HPLC assay were of HPLC grade.

Animals

Adult *Wistar albino* rats of uniform weights were procured from National Institute of Nutrition (NIN), Hyderabad. The research work was conducted under the standard guidelines and approval from the Institutional Animal Ethics Committee (Approval No. I/9/14, Dated 27.11.2014).

Experimental design

A total of 32 rats were equally divided into four groups for single dose kinetics of atorvastatin. Group 1 was maintained as healthy control without hepatotoxicity. Groups 2, 3 and 4 were administered with acetaminophen (500 mg/Kg *po* once daily for 3 days) to induce hepatotoxicity. Subsequently, group 2 was treated with distilled water (solvent, 5 ml/kg *po* once daily), group 3 with silymarin (25 mg/Kg *po* once daily) and group 4 with rutin (20 mg/Kg *po* once daily) from day 4 to day 14. Atorvastatin (10 mg/kg *po* once) was administered in all the groups after 24h post-completion (on day 15 of the experiment) of above treatment schedule and blood was collected at predetermined intervals (0.25, 0.5, 1, 3, 6, 12 and 24 hr) from the retro-orbital plexus post administration of atorvastatin. All the drugs were administered as suspensions in distilled water.

HPLC assay of atorvastatin in rat plasma

To the plasma samples (200 µl), equal volume of phosphate buffer (pH 7.0) was added and mixed well and then atorvastatin was extracted by liquid-liquid extraction technique by using methanol and phosphate buffer mixed plasma samples (1:4 ratio). The clear organic phase

Table 1: Plasma concentrations (ng.ml^{-1}) of atorvastatin after single oral administration of atorvastatin (10 mg/kg) in different groups of rats

Time (h)	Group 1 Normal control	Group 2 PCM control	Group 3 PCM +Silymarin	Group 4 PCM + Rutin
0.25	22.14±0.71	35.5±1.38	24.85±0.56	27.21±0.71
0.5	71.21±1.19	77.56±0.76	71.1±0.9	72.83±1.11
1	128.57±1.79	148.59±1.59	130.19±1.31	131.68±1.07
3	134.32±0.91	154.82±1.73	135.63±0.78	136.96±0.84
6	70.35±1.09	91.61±0.98	71.49±2.12	82.85±1.52
12	34.95±0.65	55.32±0.55	36.82±0.79	42.05±0.78
24	4.61±0.21	13.91±0.55	5.72±0.22	8.68±0.9

Values are Mean ± SE (n=8)

Table 2: Plasma pharmacokinetic parameters of atorvastatin after single oral administration of atorvastatin (10 mg/kg) in different groups of rats

Parameters	Group 1 Normal control	Group 2 PCM control	Group 3 PCM + Silymarin	Group 4 PCM + Rutin
β (h^{-1})	0.15±0.00	0.11±0.00***	0.14±0.0	0.12±0.0**
$t_{1/2}\beta$ (h)	4.41±0.07	6.23±0.09***	4.72±0.06	5.35±0.19**
AUC_{0-t} ($ng.h.mL^{-1}$)	1195.59±5.84	1604.4±13.23***	1222.12±10.06	1344.56±22.31***
$AUC_{0-\alpha}$ ($ng.h.mL^{-1}$)	1225.05±5.64	1794.92±45.87***	1261.16±9.1	1412.45±31.76**
$AUMC_{0-t}$ ($ng.h^2.mL^{-1}$)	7578.31±81.33	11644.24±191.88***	7925.74±66.6	9090.66±357.96**
$AUMC_{0-\alpha}$ ($ng.h^2.mL^{-1}$)	8473.31±114.84	17988.84±1902.19***	9129.40±50.05	11252.55±671.60
MRT (h)	6.91±0.07	9.96±0.77***	7.23±0.03	7.94±0.29
V_{dss} ($L.kg^{-1}$)	0.05±0.00	0.05±0.00	0.05±0.0	0.05±0.0
Cl_{β} ($L.kg^{-1}.h^{-1}$)	0.008±0.00	0.005±0.00***	0.007±0.00	0.007±0.00
C_{max} ($ng.mL^{-1}$)	134.32±0.91	154.82±1.73***	135.63±0.78	137.24±0.72
t_{max} (h)	3	3	3	3

Values are Mean ± SE (n=8); p< 0.05(*), p< 0.01(**), p< 0.001 (***) in comparison to Group 1 (unpaired 't'test with Welch's correction using 'Instat' software).

was separated, filtered through 0.45µm syringe filter (Millipore; Millex-HN) and evaporated to dryness at 45^0C. The filtrate was reconstituted in 40µl methanol and 20µl of filtrate was injected manually into HPLC system (Shimadzu – LC20AT) with dual wavelength UV detector (SPD-20A). The chromatographic column was C_{18} (Phenomenax®, USA; pore diameter 100±10A°, 250×4.60 mm) coated with 0.5µ silica gel.

The Mobile phase used was an isocratic solution of 0.1 mM ammonium acetate: acetonitrile (50:50), which was filtered through 0.2 µm nylon filter paper (Pall Corporation, India). The flow rate of the mobile phase was maintained @ 1 ml/min. The peak was detected at 240 nm at 6.5min after injection (Fig. 1). Peak areas of the standard plasma samples were plotted against respective known concentrations of plasma atorvastatin to obtain a linear regression line (Fig. 2).

Pharmacokinetic analysis

The plasma concentration-time profile of atorvastatin obtained for four groups in the present study was utilized for calculating pharmacokinetic parameters in rats with a linear interactive program for personal computer software (PK solver 2.0 developed by Zhang *et al*., 2010).

Statistical analysis

All data were expressed as mean ± SE. Difference in pharmacokinetic data between groups was analyzed using unpaired 't' test with Welch's correction using 'Instat' software. The level of significance was p< 0.05.

RESULTS AND DISCUSSION

Cytochrome P450 belongs to the group of isoenzymes metabolize the several parent drug into the reactive metabolites during biotransformation (Machery and Dansette, 2008; Attia *et al*., 2010). CYP3A4 is the most abundant cytochrome P450 isoform in the liver and is responsible for the metabolism of more than 60% of clinically prescribed drugs and accounts for the large number of documented drug interactions (Wilkinson, 1996). In this study, we assessed the functional status of CYP3A4 enzyme in hepatotoxicity model by estimating the plasma concentration and pharmacokinetic parameters of CYP3A4 substrate atorvastatin co–administered with silymarin and rutin.

The mean plasma concentrations (C_{max}) of atorvastatin in different groups of rats as a function of time in comparison to control rats were estimated. C_{max} of atorvastatin was reached at 3h and gradually declined at regular intervals in all the groups. Acetaminophen induced hepatotoxicity

results in inability of the body to metabolize atorvastatin, which might have resulted in increased half-life ($t_{1/2\beta}$) and Mean Residence Time (MRT), reduced total body clearance (Cl_{β}) and elimination rate constant (β) of atorvastatin in the PCM group. *In vitro*, atorvastatin is also a substrate of the intestinal P-glycoprotein (P-gp) efflux transporter, organic anion-transporting polypeptide (OATP) C and H+-monocarboxylic acid co-transporter. P-gp pumps the drug back into the intestinal lumen during drug absorption (Konig *et al*., 2013) and hence reduces the clearance of the drug.

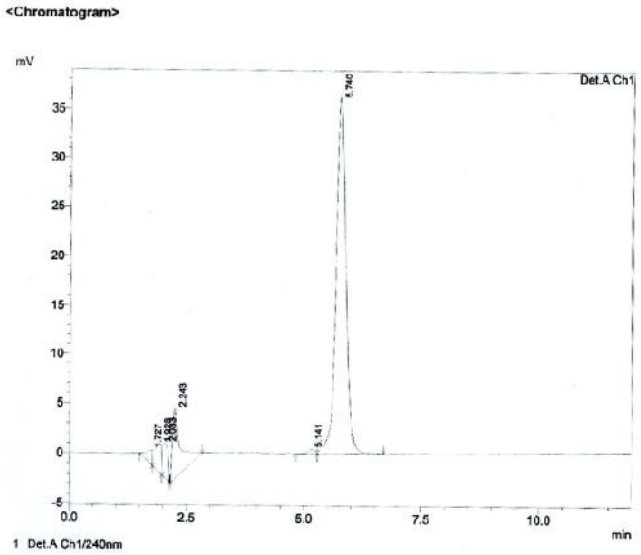

Fig. 1: Chromatogram of atorvastatin standard in plasma

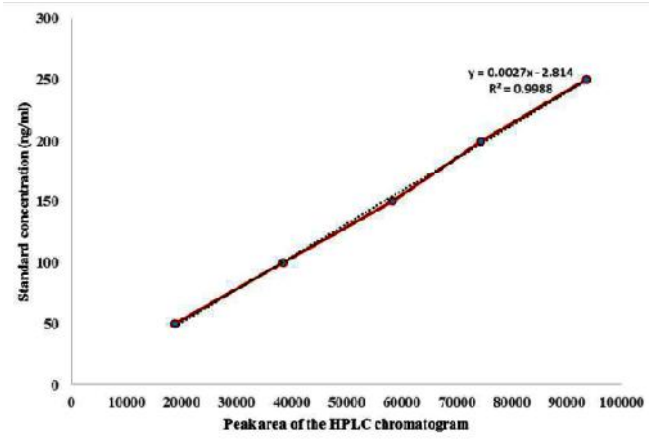

Fig. 2: Calibration curve of atorvastatin

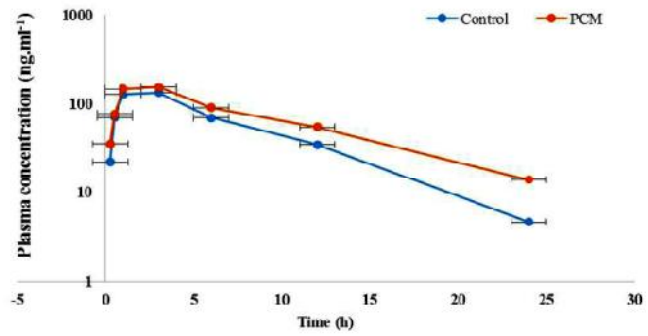

Fig. 3: Plasma atorvastatin concentrations ($ng.ml^{-1}$) in acetaminophen (PCM) treated group in comparison with control after single oral administration of atorvastatin (10 mg/kg)

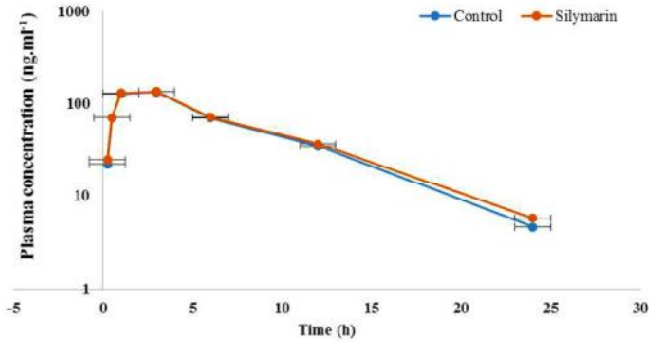

Fig. 4: Plasma atorvastatin concentrations ($ng.ml^{-1}$) in silymarin treated group in comparison with control after single oral administration of atorvastatin (10 mg/kg)

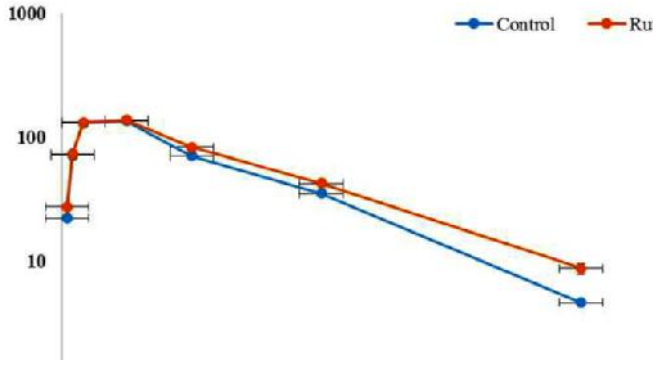

Fig. 5: Plasma atorvastatin concentrations ($ng.ml^{-1}$) in rutin treated group in comparison with control after single oral administration of atorvastatin (10 mg/kg)

C_{max} and area under the plasma concentration time curve (AUC_{0-t}) of atorvastatin was significantly increased in

PCM induced hepatotoxic control rats, which may be due to the altered metabolism of atorvastatin in the liver owing to the functional disturbance of CYP3A4. These results are in agreement with findings of Kantola *et al*. (1998) (Table 1 and 2; Fig. 3)

Rise in Cl_{β}, and fall in C_{max}, $t_{1/2\beta}$, MRT and AUC under the different time intervals of atorvastatin in silymarin treated group may be due to restoration of liver to normal when compared to that of PCM toxic group. Restoration of the liver is supported by the prevention of glutathione depletion, anti-oxidant potential and free radical scavenging properties of silymarin. These results are supported by the recent findings of Hau *et al*. 2010, Sherif and Al-Gayyar, 2013 and Feitag *et al*. 2015 (Table 2; Fig. 4).

C_{max}, $t_{1/2\beta}$ and MRT of atorvastatin in rutin treated group was significantly increased when compared to normal control group and was comparable with that of PCM treated group. Cl_{β} and AUC under different time intervals were similar to that of PCM treated group may be due to the incomplete amelioration of hepatotoxicity. The rutin is commonly used as an effective scavenger of the free radical formation and stimulation of cellular enzymatic antioxidants (Mirani *et al*., 2012). Rutin might inhibit cytochrome P450 isoenzymes and contribute favourably toward hepatoprotection (Reddy *et al*., 2016). The hepatoprotective potential of rutin is relatively lesser as compared to silymarin treated group. Among both the drugs used in the study, the silymarin-treated group 3 revealed better metabolic profile of CYP3A4 as compared to rutin-treated group 4 (Table 2; Fig. 5).

CONCLUSION

The present study concluded that the pharmacokinetic profile of atorvastatin metabolism was altered in PCM treated toxic group, which was reflected in an increase in C_{max}, $AUC_{0\text{-}t}$, $AUMC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and $AUMC_{0\text{-}\infty}$ that eventually resulted in prolonged $t_{1/2\beta}$, MRT and decrease in total body clearance. Rutin could not revive the metabolic functioning of CYP3A4 in comparison to silymarin.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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