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# Pharmacokinetic analysis of atorvastatin against experimental hepatotoxicity with special reference to CYP3A4 functioning in rats

**M. Anudeep Reddy, B. Kala Kumar, G. Boobalan*, M. Vijay Kumar, M. Kasi Reddy, C.S.V. Satish Kumar and A. Gopala Reddy**
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad – 500 030, India.
*For Correspondence - bhupalvets@gmail.com

**Abstract**
**Aim**: To assess the functional status of CYP3A4 substrate in experimental induced hepatotoxicity treated with N-acetyl L-cysteine and Green tea extract for a period of 14 days in rats.

**Materials and methods**: Twenty four *Wistar* rats were divided into four groups of 6 rats each and induced hepatotoxicity with acetaminophen (500 mg/Kg *po* once daily for 3 days) in 3 groups. Group 1: Normal control, Group 2, 3 and 4 was administered distilled water (5 ml/kg po), N-acetyl L-cysteine (NAC; 300 mg/Kg *po* once daily) and Green tea extract (GTE; 100 mg/Kg *po* once daily), respectively subsequently for 11 days from the last dose of acetaminophen. Atorvastatin (10 mg/kg *po*) was administered on 15$^{th}$ day (24 h after conclusion of treatment schedule) in all the groups. The PK studies were conducted in order to evaluate the CYP3A4 activity using the specific substrate atorvastatin in all the groups.

**Results**: $C_{max}$, $t_{1/2}$, $AUC_{0-t}$ and MRT of groups 2 and 4 were significantly ($p<0.05$) increased, while the total body clearance was significantly ($p<0.05$) decreased compared to normal control after single dose administration of atorvastatin. The kinetic profile of NAC-treated group 3 was comparable to group 1.

**Conclusion**: All the pharmacokinetic parameters of atorvastatin revealed similar values when hepatotoxic control was compared to Green tea extract treated group, while N-acetyl L-cysteine treated group revealed significant alterations in the kinetic profile that support the functional status of CYP3A4 and hence suggesting hepatoprotective potential of NAC.

**Key words**: Acetaminophen, Atorvastatin, CYP3A4, Green Tea, N-Acetyl L-cysteine

## Introduction

The pathogenesis of drug-induced hepatotoxicity accounts for the withdrawal of considerable amount of drugs from the market. In present scenario, drug induced hepatic injury is the common cause of acute and chronic liver diseases (1).

Acetaminophen (N-acetyl-p-aminophenol/ APAP), via cytochrome P450 enzyme system (CYP3A4) bioactivated to a reactive metabolite, N-acetyl-para benzoquinone imine (NAPQI) in liver. NAPQI covalently react with proteins and nucleic acids deplete GSH and potentially cause fatal centrilobular hepatic necrosis producing dose-dependent hepatotoxicity (2). The most reliable and commonly employed hepatotoxicity model to test the hepatoprotective potential of drugs is the one induced by acetaminophen (3).

Statins are primarily metabolized by CPY3A4 and co-administration of statins with drugs that inhibit CPY3A4 isoenzyme potentially increase the risk for adverse effects as the metabolism of statins gets slowed down (4).

N-acetyl-L-cysteine (NAC) is a sulfur based

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amino acid acts against oxidative stress and prevents damage to cells (5). NAC, the acetylated precursor of the amino acid L-cysteine and reduced glutathione scavenge free radicals, stimulates glutathione synthesis, enhance glutathione-S-transferase activity, and promotes liver detoxification by inhibiting xenobiotic biotransformation (6).

Green tea (*Camellia sinensis*) is rich in polyphenolic compounds that have several therapeutic and antioxidant properties (7). Administration of green tea in streptozotocin-induced diabetic rats protected liver damage due to its antioxidant property (8).

The aim of our investigation was to investigate the metabolic function of CYP3A4 under the influence of Acetaminophen-induced hepatic damage and the hepatic regeneration induced by the drugs in study (NAC and GTE) by assessing the pharmacokinetics of the specific substrate atorvastatin in rats.

**Materials and methods**

Acetaminophen (Himedia, India), Green Tea extract (Lipton, India) and N-acetyl L-cysteine (SRL, India) were administered in distilled water.

***Animals*** *:* Male *Wistar albino* rats weighing around 200-250 g were procured from Sanzyme Pvt. Ltd., Hyderabad, India. Institutional Animal Ethics Committee has approved the experimental protocol (Approval No. I/10/14 Dated 27.11.2014). Hepatotoxicity was induced in rats with oral administration of acetaminophen for 3 consecutive days (9).

***Experimental design*** *:* The study was carried out on 24 rats that were randomly divided into four groups comprising of 6 rats in each group for single dose kinetics of atorvastatin following pre-treatment with the test and standard drugs.

Group 1: Normal control.

Group 2: Acetaminophen (500 mg/Kg *po* once daily for 3 days) and distilled water (5 ml/kg po) were administered subsequently for 11 days from the last dose of acetaminophen.

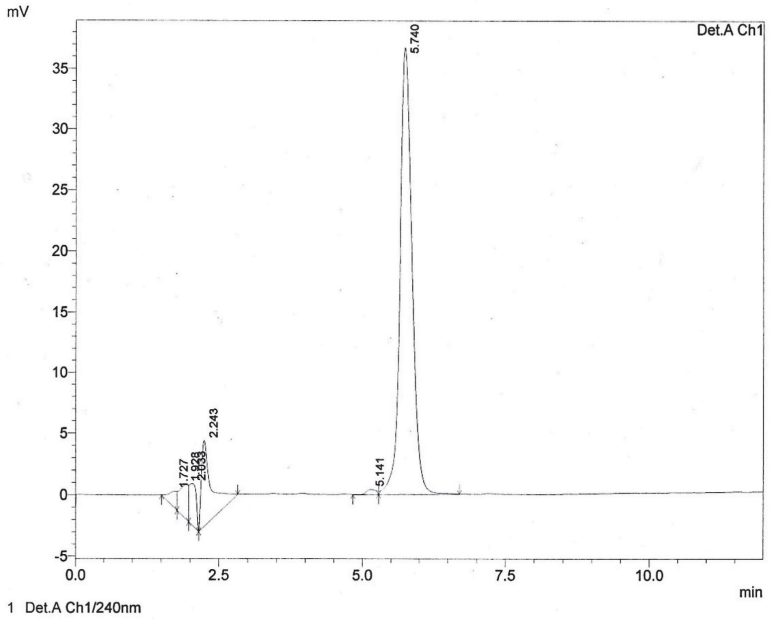
Group 3: Acetaminophen (as in group 2) and N-acetyl L-cysteine (300 mg/Kg *po* once daily) were administered subsequently for 11 days from the last dose of acetaminophen.

Group 4: Acetaminophen (as in group 2) and Green tea extract (100 mg/Kg *po* once daily) were administered subsequently for 11 days from the last dose of acetaminophen.

On the day 15 (24 h after conclusion of treatment schedule), atorvastatin was administered orally @ 10 mg/kg body weight in all the groups.

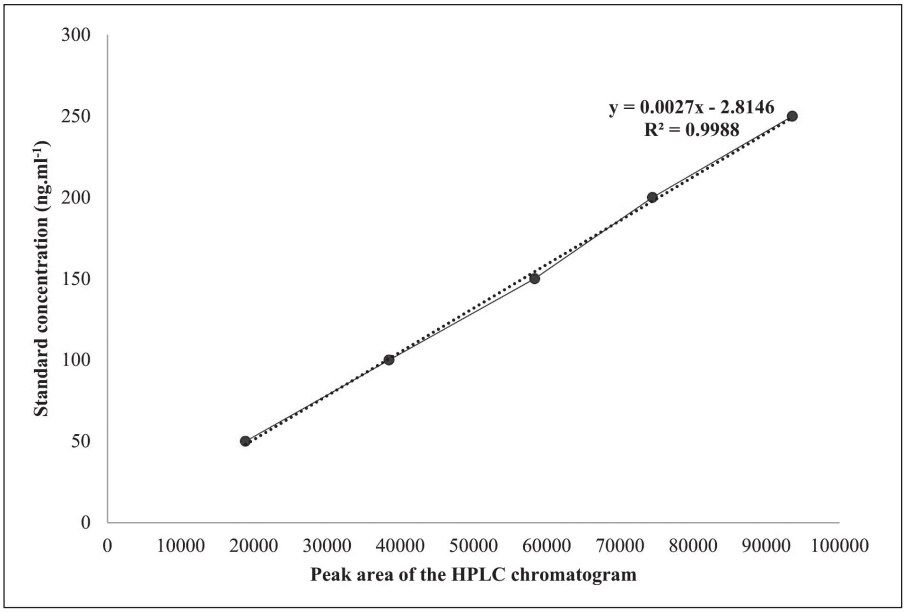
***Blood collection for PK studies :*** Blood (approximately 500 µl) was collected from retro orbital plexus at 0.25, 0.5, 1, 3, 6, 12 and 24 h post-administration of atorvastatin in the heparinized vials and the plasma was separated and stored at -20$^0$C till further analysis.

***HPLC assay of atorvastatin in rat plasma :*** To the plasma samples (200 ul), 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 (1:4 ratio). The clear organic phase was separated, filtered through 0.45µm syringe filter (Millipore; Millex-HN) and evaporated to dryness at 45$^0$C. The filtrate was reconstituted in 40ul methanol and 20ìl 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 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).




x axis = min, y axis = mV

**Fig. 1.** Chromatogram of atorvastatin standard in plasma




x axis = Peak area of the HPLC chromatogram, y axis = Standard concentration (ng.ml-)

**Fig. 2.** Calibration curve of atorvastatin

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***Pharmacokinetic analysis :*** The plasma concentration-time profile of atorvastatin obtained for four groups in the present study were utilized for calculating pharmacokinetic parameters in rats with a linear interactive programme for personal computer software (PK solver 2.0 developed by Zhang *et al*., 2010 (10)).

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

**Results**

A mean peak plasma concentration ($C_{max}$) of 134.32 ± 0.91 ng $ml^{-1}$ was achieved at 3h and gradually declined to 4.61±0.21 ng ml-1 at 24 h in group 1. Non-compartmental analysis of plasma drug concentrations yielded the mean values for area under plasma drug concentration curve ($AUC_{0-t}$), time to peak plasma concentration, ($t_{max}$), elimination rate constant (â), mean elimination half-life ($t_{1/2â}$), mean residence time (MRT), volume of distribution at steady state ($V_{dss}$) and total body clearance ($CL_â$) were 1195.59 ± 5.84 ng.h.$ml^{-1}$, 3h, 0.15 ± 0.00 $h^{-1}$, 4.41 ± 0.07 h, 6.91 ± 0.07 h, 0.05 ± 0.00 L/kg and 0.008 ± 0.00 L. $kg^{-1}$. $h^{-1}$, respectively in group 1 (Table 1 and 2).

The maximum plasma concentration ($C_{max}$) was significantly ($p<0.05$) increased in group 2 (154.82±1.73 ng.$ml^{-1}$) when compared to group 1 (134.32±0.91 ng.$ml^{-1}$). Similarly, the $AUC_{0-t,}$ $AUC_{0-"}$, $AUMC_{0-t}$ and $AUMC_{0-"}$ values of groups 2 and 4 were significantly ($p<0.05$) increased when compared to group 1. The half-life and MRT were significantly ($p<0.05$) increased in groups 2 (6.23±0.09 h and 9.96±0.77 h) and group 4 (5.79±0.12 h and 8.64±0.17 h), respectively. The total body clearance was significantly ($p<0.05$) decreased in group 2 (0.005±0.00 L.$kg^{-1}$.$h^{-1}$) and group 4 (0.006±0.01 L.$kg^{-1}$.$h^{-1}$) when compared to group 1 (0.008±0.00 L.$kg^{-1}$.$h^{-1}$). The pharmacokinetic profile of group 3 was significantly ($p< 0.05$) comparable to group 1 (Table 1 and 2; Fig. 3, 4 and 5).

**Discussion**

In this study we assessed the functional status of CYP3A4 enzyme in experimental induced hepatotoxicity by estimating the plasma concentration and pharmacokinetic parameters of CYP3A4 substrate atorvastatin in normal rats, hepatotoxic control rats and in treated groups.

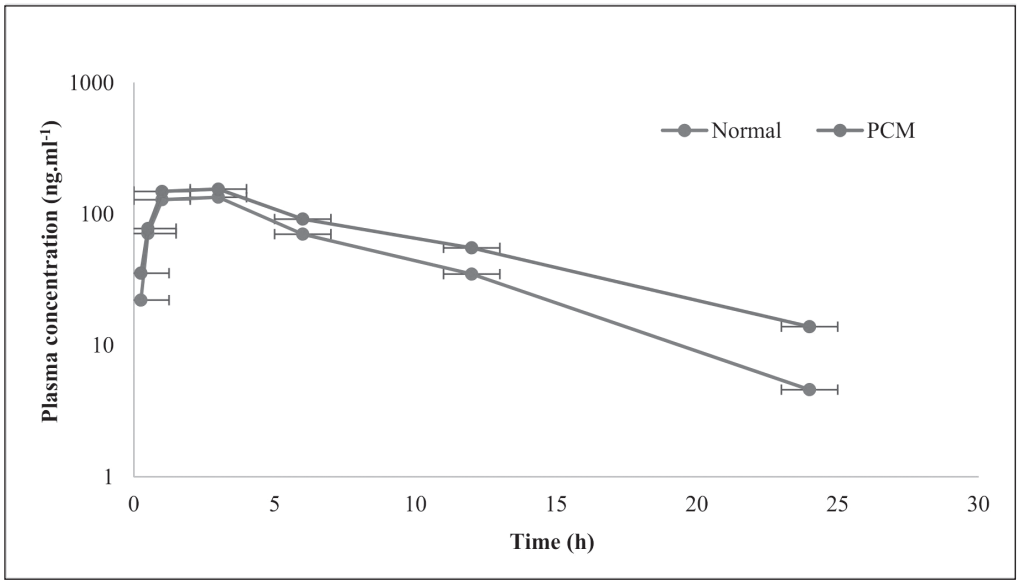
N – acetyl-para-benzoquinoneimine (NAPQI), a toxic metabolite of Acetaminophen, binds with cell macromolecules like proteins and lipids, and causes generation of ROS in liver and kidney, which may be responsible for toxic effects (11). The maximum plasma concentration ($C_{max}$) and $AUC_{0-t}$ of atorvastatin 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. The plasma concentration of atorvastatin may either increase or decrease after administration of CYP3A4 inhibitors (12) or inducers, respectively (13).

Acetaminophen induced hepatotoxicity results in inability of body to metabolize atorvastatin, which might have resulted in increased $t_{1/2â}$ and MRT of atorvastatin in the PCM group. Atorvastatin is also a substrate of the intestinal P-glycoprotein efflux transporter, which pumps the drug back into the intestinal lumen during drug absorption (14) and hence reduces the clearance of the drug.

$C_{max}$ and $AUC_{0-t}$ of atorvastatin was significantly decreased in NAC treated group may be due to restoration of liver to normal when compared to that of PCM toxic group. Atorvastatin, when administered in its active acid form, undergoes extensive first pass metabolism mainly by CYP3A4 in the liver (15). Restoration of liver to normal might have favoured the drug metabolism by CYP3A4, which resulted in decreased $C_{max}$ in NAC treated group.

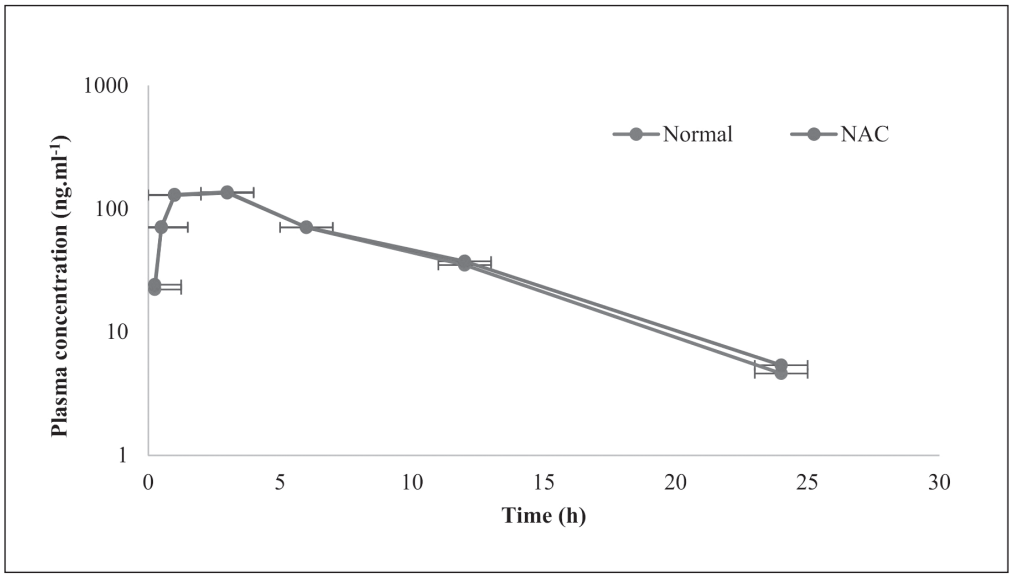
$C_{max}$, $t_{1/2â}$ and MRT of atorvastatin in GTE group was significantly increased when compared to normal control group and was comparable with that of PCM treated group. Catechins and flavonoids present in green tea

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




x axis = Time (h), y axis = Plasma concentration (ng.ml$^{-1}$)

**Fig. 4.** Plasma atorvastatin concentrations (ng.ml-1) in N-acetyl-L-cysteine (NAC) treated group in comparison with control after single oral administration of atorvastatin (10 mg/kg)




x axis = Time (h), y axis = Plasma concentration (ng.ml$^{-1}$)

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**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+NAC | Group 4 PCM+GTE |
|---|---|---|---|---|
| 0.25 | 22.14±0.71 | 35.5±1.38 | 27.21±0.71 | 29.76±0.30 |
| 0.5 | 71.21±1.19 | 77.56±0.76 | 72.83±1.11 | 76.69±0.99 |
| 1 | 128.57±1.79 | 148.59±1.59 | 131.68±1.07 | 136.37±3.72 |
| 3 | 134.32±0.91 | 154.82±1.73 | 136.96±0.84 | 141.09±2.94 |
| 6 | 70.35±1.09 | 91.61±0.98 | 82.85±1.52 | 87.24±1.15 |
| 12 | 34.95±0.65 | 55.32±0.55 | 42.05±0.78 | 48.26±0.88 |
| 24 | 4.61±0.21 | 13.91±0.55 | 8.68±0.9 | 10.88±0.49 |

*Values are Mean ± SE (n=6)

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

| Parameter | Group 1 Normal control | Group 2 PCM control | Group 3 PCM+NAC | Group 4 PCM+GTE |
|---|---|---|---|---|
| $\beta$ ($h^{-1}$) | 0.15±0.00 | 0.11±0.00*** | 0.14±0.00 | 0.11±0.00*** |
| $t_{1/2}\beta$ (h) | 4.41±0.07 | 6.23±0.09*** | 4.62±0.08 | 5.79±0.12*** |
| $AUC_{0-t}$ (ng.h.mL$^{-1}$) | 1195.59±5.84 | 1604.4±13.23*** | 1214.69±14.28 | 1451.7±18.35*** |
| $AUC_{0-á}$ (ng.h.mL$^{-1}$) | 1225.05±5.64 | 1794.92±45.87*** | 1250.76±13.03 | 1543±20.47*** |
| $AUMC_{0-t}$ (ng.h$^2$.mL$^{-1}$) | 7578.31±81.33 | 11644.24±191.88*** | 9090.66±357.96** | 10380.71±178.26*** |
| $AUMC_{0-á}$ (ng.h$^2$.mL$^{-1}$) | 8473.31±114.84 | 17988.84±1902.19*** | 8928.58±95.83 | 13338.66±366.43* |
| MRT (h) | 6.91±0.07 | 9.96±0.77*** | 7.13±0.04 | 8.64±0.17* |
| $V_{dss}$ (L.kg$^{-1}$) | 0.05±0.00 | 0.05±0.00 | 0.05±0.00 | 0.05±0.00 |
| $Cl_á$ (L.kg$^{-1}$.h$^{-1}$) | 0.008±0.00 | 0.005±0.00*** | 0.007±0.00 | 0.006±0.01*** |
| $C_{max}$ (ng.mL$^{-1}$) | 134.32±0.91 | 154.82±1.73*** | 136.38±0.27 | 141.09±2.94 |
| $t_{max}$ (h) | 3 | 3 | 3 | 3 |

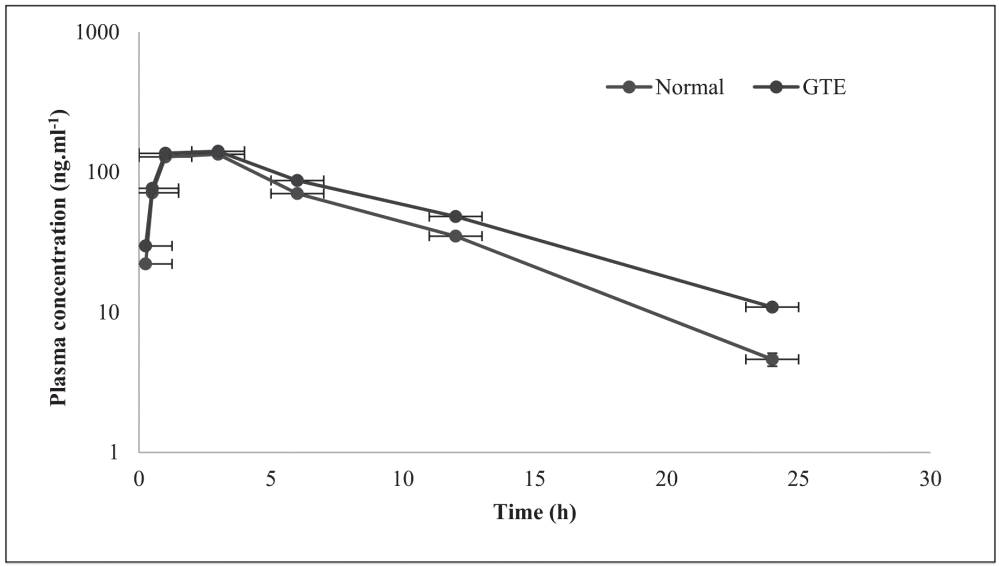
Values are Mean ± SE (n=6); p< 0.05(*), p< 0.01(**), p< 0.001 (***) in comparison to Group 1 (unpaired 't' test with Welch's correction using 'Instat' software)

reduce oxidative stress by effectively scavenge reactive oxygen species and by enhancing the body's antioxidant defences (16). But, the hepatoprotective potential of GTE is comparatively lower when compared to NAC treated group. Among both the drugs in study, the NAC-treated group 3 revealed better metabolic profile of CYP3A4 as compared to Green tea extract-treated group 4.

## Conclusion

The present study concluded that pharmacokinetics of atorvastatin metabolism was altered in PCM treated toxic group, which was reflected in increase in $C_{max}$, $AUC_{0-t}$, $AUMC_{0-t}$, $AUC_{0-"}$ and $AUMC_{0-"}$ that eventually resulted in prolonged $t_{1/2â}$, MRT and decrease in total body clearance. Green tea extract could not revive the metabolic functioning of CYP3A4 in comparison to N-acetyl L-cysteine.

**Fig. 5.** Plasma atorvastatin concentrations (ng.ml-1) in Green tea extract (GTE) treated group in comparison with control after single oral administration of atorvastatin (10 mg/kg)




x axis = Time (h), y axis = Plasma concentration (ng.ml$^{-1}$)

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**Competing Interests**

Authors declare that they have no competing interests.

**References**

1. Kaplowitz, N. (2004). Drug-induced liver injury. Clinical Infectious Diseases, 38 (Suppl 2): 44-48.
2. Zhang, L., Gavin, T., Geohagen, B.C., Liu, Q., Downey, K.J. and LoPachin, R.M. (2013). Protective properties of 2-acetylcyclopentanone in a mouse model of acetaminophen hepatotoxicity. Journal of Pharmacology and Experimental Therapeutics, 346(2): 259–269.
3. Olaleye, M.T, Amobonye, A.E., Komolafe, K., Akinmoladun, A.C. (2014). Protective effects of *Parinari curatellifolia* flavonoids against acetaminophen-induced hepatic necrosis in rats. Saudi Journal of Biological Sciences, 21(5): 486-492.
4. Rowan, C.G. (2010). Clinical importance of the drug interaction between statins and CYP3A4 inhibitors. Publicly accessible Penn Dissertations. Paper 283.
5. Oh, S.-I., Park, J.-K. and Park, S.-K. (2015). Lifespan extension and increased resistance to environmental stressors by N-Acetyl-L-Cysteine in *Caenorhabditis elegans*. Clinics, 70(5): 380-386.
6. Ghezzi, P. (2011). Role of glutathione in immunity and inflammation in the lung. International Journal of General Medicine, 4: 105-113.

7. Gonbad, R.A., Afzan, A., Karimi, E., Sinniah, U.R. and Swamy, M.K. (2015). Phytoconstituents and antioxidant properties among commercial tea (*Camellia sinensis* L.) clones of Iran. Electronic Journal of Biotechnology, 18: 433–438.

8. Abolfathi, A.A., Mohajeri, D., Rezaie, A. and Nazeri, M. (2012). Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats. Evidence-Based Complementary and Alternative Medicine, 2012: 740671, 10 pages. Doi:10.1155/2012/740671.

9. Sreedevi, C.D., Latha, P.G., Ancy, P., Suja, S.R., Shyamal. S., Shine, V.J., Sini, S., Anuja, G.I. and Rajasekharan, S. (2009). Hepatoprotective studies on *Sida acuta* Burn. f. Journal of Ethnopharmacology, 124: 171–175.

10. Zhang, Y., Huo, M., Zhou, J. and Xie, S. (2010). PK solver: An add in program for pharmacokinetic and pharmacodynamics data analysis in Microsoft excel. Computer methods and program in Biomedicine, 99: 306-314.

11. Attia, S.M. (2010). Deleterious effects of reactive metabolites. Oxidative Medicine and Cellular Longevity, 3(4): 238–253.

12. Borders-Hemphill, V. (2009). Concurrent use of statins and amiodarone. The Consultant Pharmacist, 24: 372–379.

13. Lam, S., Partovi, N., Ting, L.S. and Ensom, M.H. (2008). Corticosteroid interactions with cyclosporine, tacrolimus, myco-phenolate, and sirolimus: fact or fiction? Annals of Pharmacotherapy, 42:1037–1047.

14. König, J., Müller, F. and Fromm, M.F. (2013). Transporters and drug-drug interactions: important determinants of drug disposition and effects. Pharmacological Reviews 65(3): 944-966.

15. Chong, P.H., Seeger, J.D. and Franklin, C. (2011). Clinically relevant differences between the statins: implications for therapeutic selection. American Journal of Medicine, 111: 390–400.

16. Higdon, J.V. and Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism and antioxidant functions. Critical reviews in food science and nutrition, 43: 89-143.