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Pharmacokinetic profile of atorvastatin in experimental hepatotoxic untreated and treated rats with n-acetyl cysteine and *Costus pictus*

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Abstract
Objective: To study the pharmacokinetic profile of atorvastatin in experimental hepatotoxic untreated and treated rats with N-acetyl cysteine and *Costus pictus* with special reference to assess the functional status of CYP3A4.
Materials and Methods: Twenty *Sprague dawley* rats of 3 months age were divided into 4 groups, comprising of 5 rats in each group. Acetaminophen (APAP) @ 500 mg/kg BW was administered orally to all the groups except group 1 from day 1 to 3. Group 1 was maintained as normal control. Group 2 was subsequently administered with distilled water (p/o) from day 4 to 17 and was considered as toxic control. Groups 3 and 4 were administered (p/o) with N-Acetyl cysteine @ 300 mg/kg BW and *Costus pictus* aqueous extract @ 500 mg/kg BW, respectively from day 4 to 17. Atorvastatin was administered on day 18 as single oral dose in all the groups and blood samples were drawn at specified intervals for pharmacokinetic analysis.
Results: Atorvastatin metabolism was altered in APAP treated toxic group, which was reflected in increase in C_{max}, AUC_{0-t}, $AUMC_{0-t}$, $AUC_{0-\infty}$ and $AUMC_{0-\infty}$ that eventually resulted in prolonged $t_{1/2\beta}$, MRT and decrease in clearance. Among the treated groups, group 3 (N-acetyl cysteine) revealed better metabolic profile of CYP3A4 as compared to group 4 (*Costus pictus* extract).
Conclusion: The treated groups revealed significant alteration in the pharmacokinetic profile that supports the functional status of CYP3A4 and when groups 3 and 4 are compared, the pharmacokinetic profile was found better in group 4 in comparison to group 3 suggesting that N-acetyl cysteine is more potent than *Costus pictus* in the hepatoprotective activity.

Keywords: Atorvastatin, acetaminophen, N-acetyl cysteine, *Costus pictus*, CYP3A4, pharmacokinetics

1. Introduction
Over the past decade, the dramatic growth in the number of new prescriptions and over-the-counter drugs has greatly improved the therapeutic armamentarium but at the expense of an increased risk of adverse drug events, in particular hepatotoxicity [1]. Acetaminophen (N-acetyl-p-aminophenol; APAP) is a commonly used medication as a pain reliever and fever reducer because of its mild non-narcotic, analgesic and antipyretic activity [2]. Acetaminophen is safe at therapeutic doses but causes acute liver damage with overdose [3].
CYP3A4 is the major P450 isoform in human liver and enterocytes that contributes appreciably in the formation of cytotoxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) at therapeutically relevant concentrations of N-acetyl-p-aminophenol (APAP) [4]. The excess N-acetyl-p-aminophenol is shunted to the CYP system, which leads to more production of N-acetyl-p-benzoquinone imine (NAPQI) and depletion of GSH resulting in retention of NAPQI in the liver, where it covalently binds to cell protein thiols altering their functions and eventually leading to acute hepatic necrosis [5].
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 CYP3A4 inhibitors prevent CPY3A4 isoenzyme from metabolising statins [6].
N-acetyl cysteine (NAC) is a potent antioxidant and sulfur-based amino acid and an effective antidote for acetaminophen-induced hepatotoxicity and is beneficial against reactive oxygen species (ROS) generation, mitochondrial dysfunctions and in mitochondrial dependent and independent apoptotic cell death in cancer [7]. N-acetyl cysteine also has anti-inflammatory, antioxidant, inotropic, and vasodilating effects, which may further benefit the patient [2].

Plants produce significant amount of antioxidants to control oxidative stress, which was caused by sunbeams and oxygen [8]. *Costus pictus* commonly called insulin plant is one of the folk medicines used for the treatment of diabetes mellitus because of its antidiabetic property and is originated from Mexico [9]. The leaves of *Costus pictus* have good antioxidant, anti-inflammatory, hypo-glycaemic properties [10]. The plant *Costus pictus* has highest therapeutic potential as it is rich in phytoconstituents and the active ingredients are alkaloids, steroids, terpenoids, glycosides, tannins, saponins, phenols, flavonoids and proteins [11].

The objective of the study was to assess the functional status of CYP3A4 enzyme in experimentally induced hepatotoxicity by estimating the plasma concentration and pharmacokinetic parameters of CYP3A4 substrate (atorvastatin) in normal, toxic control and treated groups.

2. Materials and Methods

Acetaminophen (Himedia, Mumbai), N-acetyl-L-cysteine (Sisco Research Laboratories Pvt. Ltd., Mumbai), *Costus pictus* leaves (Gour Agro Farms, Rock Garden, Tamilnadu) and Atorvastatin (Storvas®10 brand, Ranbaxy Laboratories) were administered in distilled water.

2.1 Experimental Design

A total of 20 *Sprague dawley* rats of 3 months age were procured from Jeeva life sciences, Hyderabad, India. Rats were divided into 4 groups, comprising of 5 rats in each and kept in poly propylene cages and maintained with 12 hour dark/light cycle at college animal house. All the rats were provided with feed and water *ad libitum* throughout the experiment. The experimental protocol was accepted by the Institutional Animal Ethics Committee (Approval No. 3/2017-SA Dated 16-5-2017). The Pharmacokinetic studies were conducted to in order to evaluate the functioning of CYP3A4 using the specific substrate atorvastatin in all the groups that were maintained as follows:

Group 1: Normal control

Group 2: Acetaminophen @ 500 mg/kg body weight (BW) once daily for 3 days, distilled water was administered subsequently for 14 days from the last dose of acetaminophen.

Group 3: Acetaminophen (as in group II) + N-Acetyl cysteine @ 300 mg/kg was administered for 14 days after the last dose of acetaminophen.

Group 4: Acetaminophen (as in group II) + *Costus pictus* aqueous extract @500 mg/kg orally administered for 14 days after the last dose of acetaminophen.

On the day 18 (24 h after conclusion of treatment schedule), atorvastatin was administered orally at the dose rate of 10 mg/kg body weight in all the groups and blood (approximately 500 µl) was collected from retro-orbital plexus at 0.25, 0.5, 1, 3, 6, 12 and 24 h into heparinized containers and plasma was separated by centrifugation at 3000 RPM for 15 min and stored at -20^{0}C till analysis.

2.2 Measurement of Plasma Atorvastatin Concentration

To the plasma sample (200 µl), an equal volume of phosphate buffer (pH 7.0) was added and mixed well and then atorvastatin was extracted by liquid–liquid extraction using methanol (1:4 ratio), following which the organic phase was separated, filtered through nylon 0.45‑µm syringe filter (Millipore; Millex‑HN) and evaporated to dryness at 45°C and then reconstituted in 40 µl of methanol; further, 20 µl of this was injected into the High‑Performance Liquid Chromatography (HPLC) system (Shimadzu LC 20AT/SPD‑20A detector). Same extraction procedure was applied for the standard samples and plasma samples to be assayed for pharmacokinetic analysis. The chromatographic column was C‑18 (Phenomenex, USA; 100A size 250 × 4.60 mm) coated with 0.5 µ 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 was adjusted to 1 ml/min. The peak was detected at 240 nm at 6.5 min after injection. Stock solutions of atorvastatin were prepared by dissolving it in methanol at a concentration of 500 µg/ml. This solution was diluted with methanol to obtain the working standard solutions in the range of 75‑5000 ng/ml. Plasma standards were prepared in the range of 7.5‑500 ng/ml by taking 100 µl of working standard solutions in a disposable tube, evaporating to residue by heating at 45°C. To the residue, 1 ml of pooled untreated plasma was added and mixed in a vortex mixer for 30 sec and used for standard curve preparation. Standard curve was prepared from working plasma standards in the range of 7.5‑500 ng/ml. After extraction of the drug, standard samples were injected into the HPLC system and peak areas were recorded and plotted against respective known concentrations of plasma atorvastatin to obtain a linear regression line (standard curve).

2.3 Pharmacokinetic analysis

Plasma concentration versus time data of atorvastatin btained for four groups in the present study were utilized for calculating various pharmacokinetic parameters in rats with a linear interactive programme for personal computer software (PK Solver, version. 2.0, developed by Zhang *et al*., 2010).

2.4 Statistical analysis

The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 21. Differences between means were tested using Duncan's multiple comparison test and significance was set at $p<0.05$.

3. Results

The mean plasma concentrations of atorvastatin in different groups of rats as function of time in comparison to control rats were presented in Table 1. Mean peak plasma concentration (C_{max}) of 135.32±0.46 ng.ml^{-1} was achieved at 3 h and it gradually declined to 4.96±0.04 ng.ml^{-1} at 24 h in group 1. In group 2, the mean peak plasma concentration was achieved at 3 h and it was significantly ($p<0.05$) higher (150.99±0.38 ng.ml^{-1}) as compared to control group 1 (Fig.1). The mean peak plasma concentration of group 3 was 137.80±0.70 ng.ml^{-1}, which was comparable with normal. The mean peak plasma concentration of group 4 was 139.36±0.32 ng.ml^{-1}, and the values of groups 3 and 4 were comparable to that of group 1 (Fig. 2 and Fig. 3).

Non-compartment analysis of plasma drug concentrations yielded the mean values for area under plasma drug concentration versus time curve (AUC_{0-t}), area under first moment curve ($AUMC_{0-t}$), maximum plasma concentration (C_{max}), time to peak plasma concentration (t_{max}), elimination

rate constant (β), mean elimination half-life ($t_{1/2\beta}$), mean residence time (MRT), volume of distribution at steady state (V_{dss}) and total body clearance (Cl_{β}) as 1197.73±2.27 ng.h.ml^{-1}, 7629.79±33.13 ng.h^2.mL^{-1}, 135.32±0.46 ng.mL^{-1}, 3 h, 0.15±0.0 h^{-1}, 4.50±0.01 h, 7.00±0.01 h, 0.05±0.0 L.kg^{-1} and 0.008±0.00 L.kg^{-1}.h^{-1}, respectively (Table 2) in group 1.

When compared to group 1 (135.32±0.46 ng.ml^{-1}) the maximum plasma concentration (C_{max}) was significantly increased in group 2 (150.99±0.38 ng.ml^{-1}). The AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-t}$ and $AUMC_{0-\infty}$ values of groups 3 and 4 were significantly ($p<0.05$) decreased when compared to group 2. The half-life was significantly decreased in groups 3 (5.45±0.04 h) and 4 (5.80±0.2 h) when compared to group 2 (6.31±0.02 h). Similarly, the MRT was significantly decreased in groups 3 (8.15±0.03 h) and 4 (8.68±0.03 h) when compared to group 2 (9.33±0.02 h). The total body clearance was significantly increased in group 3 (0.007±0.00 L.kg^{-1}.h^{-1}) when compared to group 2 (0.006±0.00 L.kg^{-1}.h^{-1}) and total body clearance of group 4 (0.006±0.00) was comparable to that of group 2.

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

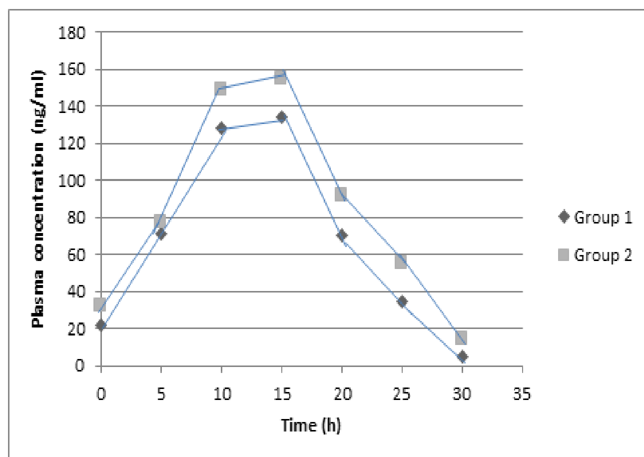
Time (h)	Group 1 Normal control	Group 2 APAP control	Group 3 APAP+NAC	Group 4 APAP+*Costus pictus*
0.25	23.55±0.46	32.58±0.44	28.15±0.38	30.42±0.38
0.5	71.03±0.28	77.95±0.30	72.88±0.31	78.25±0.37
1	127.84±0.38	148.00±0.29	132.44±0.64	132.23±0.63
3	135.32±0.46	150.99±0.38	137.80±0.70	139.36±0.32
6	71.19±0.46	90.81±0.35	83.36±0.51	88.94±0.56
12	35.21±0.38	57.73±0.60	42.61±0.21	48.49±0.36
24	4.96±0.04	13.96±0.13	9.11±0.16	10.95±0.13

Values are Mean ± SE (n=5)

Table 2: Plasma pharmacokinetic parameters of atorvastatin in different groups of rats after single oral administration of atorvastatin (10 mg.kg^{-1})

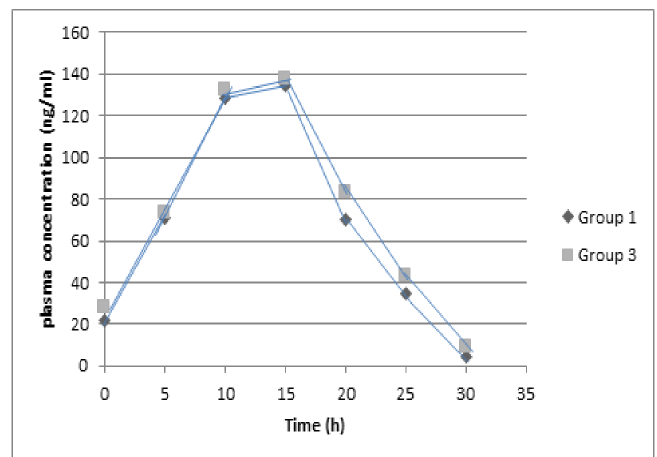
Parameter	Units	Group 1 Normal control	Group 2 APAP control	Group 3 APAP+NAC	Group 4 APAP+*Costus pictus*
β	h^{-1}	0.15±0.0^A	0.10±0.0^D	0.12±0.0^B	0.11±0.0^C
$t_{1/2}\beta$	h	4.50±0.01^D	6.31±0.02^A	5.45±0.04^C	5.80±0.2^B
AUC_{0-t}	ng.h.mL^{-1}	197.73±2.27^D	1611.89±4.42^A	1357.75±6.30^C	1453.11±4.71^B
$AUC_{0-\alpha}$	ng.h.mL^{-1}	1230.01±2.25^D	1739.10±6.17^A	1429.46±6.75^C	1545.00±3.10^B
$AUMC_{0-t}$	ng.h^2.mL^{-1}	7629.79±33.13^D	12032.06±44.66^A	9379.47±43.72^C	10477.38±53.31^B
$AUMC_{0-\alpha}$	ng.h^2.mL^{-1}	8614.53±34.33^D	16243.42±32.28^A	11665.27±85.06^C	13419.80±31.32^B
MRT	h	7.00±0.01^D	9.33±0.02^A	8.15±0.03^C	8.68±0.03^B
V_{dss}	L.kg^{-1}	0.05±0.0^A	0.05±0.0^A	0.04±0.0^B	0.05±0.0^A
Cl_{β}	L.kg^{-1}.h^{-1}	0.008±0.00^A	0.006±0.00^C	0.007±0.00^B	0.006±0.00^C
C_{max}	ng.mL^{-1}	135.32±0.46^C	150.99±0.38^A	137.80±0.70^B	139.36±0.32^B
t_{max}	h	3	3	3	3

Values are Mean ± SE (n=5); One way ANOVA (SPSS); Means with different alphabets as superscripts differ significantly ($P<0.05$).



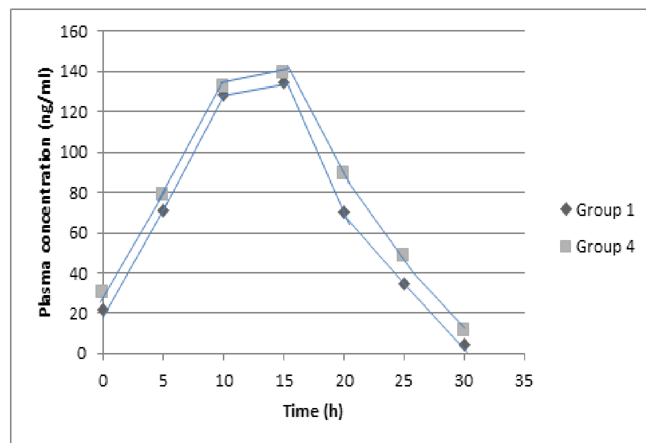
x axis = Time (h), y axis = Plasma concentration (ng/ml)

Fig 1: Plasma atorvastatin concentrations (ng.ml-1) in acetaminophen treated group 2 in comparison with control group after single oral administration of atorvastatin (10 mg/kg).



x axis = Time (h), y axis = Plasma concentration (ng/ml)

Fig 2: Plasma atorvastatin concentrations (ng.ml-1) in group 3 in comparison with control group after single oral administration of atorvastatin (10 mg/kg).



x axis = Time (h), y axis = Plasma concentration (ng/ml)

Fig 3: Plasma atorvastatin concentrations (ng.ml-1) in group 4 in comparison with control group 1 after single oral administration of atorvastatin (10 mg/kg).

4. Discussion

Area under plasma concentration versus time curve (AUC) is an important parameter and is needed for calculating the bioavailability of a drug and to determine the clearance of a drug from the body. Mean residence time (MRT) is an important pharmacokinetic parameter, which gives indication about the time for which the drug persists in the body after administration.

The maximum plasma concentration (C_{max}) of atorvastatin was significantly increased in APAP induced hepatotoxic control rats (group 2) when compared with control group 1. Similarly AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-t}$ and $AUMC_{0-\infty}$ values were significantly increased when compared to control group 1. The increase in C_{max} and AUC_{0-t} in APAP group [2] may be due to the altered metabolism of atorvastatin in the liver owing to the functional disturbance in the activity of CYP3A4. NAPQI, which is a toxic metabolite of acetaminophen, bind with cell macromolecules like proteins and lipids, and cause generation of ROS in liver and kidney, which may be responsible for toxic effects [12]. Due to damage to the liver caused by paracetamol, there may be a decreased number of hepatic cells to metabolize atorvastatin, which might have resulted in higher concentrations of atorvastatin in the blood. The $t_{1/2\beta}$ was significantly increased in group 2. Longer elimination half-life resulted due to the lowered clearance of atorvastatin in group 2. Similarly, MRT value was significantly increased in group 2. Decreased metabolism of atorvastatin, which resulted in higher concentrations of atorvastatin, might have resulted in increase of $t_{1/2\beta}$ and MRT of atorvastatin in the APAP group. The clearance (Cl_{β}) value was significantly lowered in group 2 suggesting reduced metabolism by CYP3A4. Atorvastatin is a substrate of the intestinal P-glycoprotein efflux transporter, which pumps the drug back into the intestinal lumen during drug absorption [13] and hence reduces the clearance of the drug.

The mean maximum plasma concentration (C_{max}) of atorvastatin was significantly decreased in NAC treated group when compared to APAP treated toxic group. Similarly, the AUC_{0-t}, $ACU_{0-\infty}$, $AUMC_{0-t}$, $AUMC_{0-\infty}$ values were significantly decreased when compared to APAP toxic group. The decrease in AUC_{0-t} and C_{max} in NAC treated group may be due to restoration of liver function to normal. Atorvastatin undergoes extensive first pass metabolism mainly by CPY3A4 in liver, when administered in its active form [14] so restoration of liver to normal might have favoured the drug metabolism by CYP3A4, which resulted in decreased C_{max} when compared to that of APAP toxic group.

The mean maximum plasma concentration (C_{max}) of *Costus pictus* treated group 4 was significantly decreased when compared to APAP toxic group and was comparable with that of NAC treated group. Similarly the AUC_{0-t}, $ACU_{0-\infty}$, $AUMC_{0-t}$, $AUMC_{0-\infty}$ were significantly decreased in *Costus pictus* treated group when compared to that of APAP toxic group. The $t_{1/2\beta}$ and MRT of *Costus pictus* treated group were significantly decreased when compared to APAP toxic group suggesting the improvement in liver functioning and CYP3A4 activity, which may be due to the hepatoprotective activity. Flavonoids and phenolic acids present in the leaves [15] scavenge off the free radicals, they also inhibit the generation of free radicals and hence inhibit the cellular damage too [16]. Among the treated groups, group 3 (N-acetyl cysteine) revealed better metabolic profile of CYP3A4 as compared to group 4 (*Costus pictus* extract).

5. Conclusion

The present study concludes to reveal that the atorvastatin metabolism was altered in APAP treated toxic group, which was reflected in increase in C_{max}, AUC_{0-t}, $AUMC_{0-t}$, $AUC_{0-\infty}$ and $AUMC_{0-\infty}$. Prolonged $t_{1/2\beta}$, MRT and decrease in clearance. The treated groups revealed significant alteration in the pharmacokinetic profile that supports the functional status of CYP3A4. When groups 3 and 4 were compared, the pharmacokinetic profile was found better in NAC treated group (4) in comparison to *Costus pictus* extract treated group (3) suggesting that NAC is more potent than *Costus pictus*.

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