

## Research Article



## Evaluation of Clarithromycin Availability at Intestine and Liver in Rat after Induction of Hyperlipidemia by High Fat Diet and Comparison with Non-Hyperlipidemia

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### ABSTRACT

To evaluate impact of hyperlipidemia and Atorvastatin on Clarithromycin availability at intestine and liver in presence of induced hyperlipidemia. Clarithromycin metabolism was compared presence or absence of hyperlipidemia and antipyrine treated rats. Fraction of drug escaping from the intestine ( $F_a$ ,  $F_g$ ) and Hepatic extraction ratio ( $E_h$ ) of Clarithromycin in induced hyperlipidemic rats were estimated using a plasma concentration profiles obtained simultaneously from portal as well as systemic circulation. Three groups out of five were allotted to understand Clarithromycin pharmacokinetics in rat details as follows: first two groups Clarithromycin treated in the presence and absence of hyperlipidemia and in last study group Clarithromycin treated in the reduced hyperlipidemia by treating anti-hyperlipidemic drug like Atorvastatin. First three groups were dosed up to 10 days and lipid profiles were compared day 1 and day 10. Last two groups were treated with antipyrine in presence and absence of hyperlipidemia and compared. The  $F_a$ - $F_g$  in across the conditions found to be almost similar ratio was ranging from 0.75 to 0.89. The fraction of Clarithromycin escaping from the liver ( $F_l$ ) was 0.63 in induced hyperlipidemic rat model, 0.47 and 0.49 in non-hyperlipidemic rat and reduced hyperlipidemic models, respectively. Investigated that antipyrine liver extraction ratio was significantly reduced in the presence of hyperlipidemia. Pharmacodynamic assessment like Total Cholesterol and Triglycerides levels were estimated on day 1 and day 10 following repeated once a day dose administration. It is suggested that Clarithromycin metabolism was significantly altered in presence of hyperlipidemia. Antipyrine assessment was proved and also one of the strong supportive data to conclude the Clarithromycin metabolism. This study represented valuable information on Clarithromycin pharmacokinetics for the evaluation of hyperlipidemia incidence.

**Keywords:** Bioavailability, Clarithromycin, Hepatic availability, Hyperlipidemia, Intestinal availability, Pharmacokinetics, Total cholesterol, Triglycerides.

### INTRODUCTION

There is an epidemic of overweight and metabolic syndrome worldwide and consequently there is increasing medical focus on the issue of hyperlipidemia. Studies have documented the increasing prevalence of overweight and obesity in children and adolescents, with a concomitant increase in the prevalence of cardiovascular risk factors.<sup>1</sup> Chlamydia (C) pneumonia is a common respiratory pathogen responsible for community-acquired pneumonia, sinusitis, and bronchitis.<sup>2,3</sup> It is also strongly implicated in the pathogenesis of atherosclerosis based on seroepidemiological studies,<sup>4,5</sup> histopathological findings of the organisms in atheromatous plaques,<sup>6,7</sup> the recovery of viable organisms from carotid and coronary disease arteries,<sup>8,9</sup> and various animal models. Induced vascular damage of the aorta resembling early lesions of atherosclerosis in the rabbit by C. pneumonia infection by itself<sup>10</sup> or by C. pneumonia infection in combination with a low cholesterol-enriched chow<sup>11</sup> that can enhance atherosclerosis. Clarithromycin (CLR), like all macrolides, is recommended treatment for community-acquired pneumonia and lower-respiratory-tract infection with this organism. However, there is no report of Clarithromycin on atherosclerosis in the animal model. Furthermore, the newer macrolides have anti-inflammatory activity<sup>12</sup> and studies are needed to determine whether any possible

modifying effect on the CLR pharmacokinetics in presence of atherosclerosis could be related to this liver metabolism rather than the antimicrobial effect.<sup>13</sup> A total of 11,583 CLR users are studied to evaluate hyperlipidemia (HL) reported to have a side effects when taking CLR among them 105 people have HL.<sup>14</sup> The evidence is now strong for a link between lipid abnormalities, cardiovascular disease and Clarithromycin. Hyperlipidemia is an increase in the amount of fat such as Total Cholesterol (TC) and Triglycerides (TG) in the blood. An increase of TC and TG can lead to heart disease and pancreatitis. There are several secondary causes of hyperlipidemia like liver disease, obstructive and renal disease etc. to produce abnormal lipids that may occur in adolescence.<sup>15</sup> Drug transporters as well as drug-metabolizing enzymes play a critical role in the disposition and the elimination of xenobiotics. For example, P-glycoprotein (P-gp1) is a prominent ATP-dependent efflux pump that has broad substrate specificity.<sup>16</sup> Cytochrome P450 is key members of the group of phase I enzymes metabolizing many drugs, and it plays an important role in the first-pass metabolism of drugs after oral administration. P450s are mainly expressed in the liver, intestinal wall, and kidney.<sup>17,18</sup> Lipoproteins can contain one or a variety of apolipoproteins, which regulate their metabolic functions.<sup>19</sup> In general, apolipoproteins are



involved in several physiological functions of lipoproteins such as facilitation of lipid transport, maintenance of structural integrity, and activation of certain enzymes that play key roles in lipid metabolism.<sup>20-22</sup> Hepatic triglyceride lipase, also known as hepatic lipase, is located on endothelial cells of hepatic sinusoids and several extra hepatic tissues, and is involved in hepatic uptake of triglycerides and phospholipids from chylomicrons.<sup>23</sup> There were no significant safety concerns and hyperlipidemia appeared to be the primary limiting factor. No studies have been initiated to know the CLR availability at intestine and liver in induced hyperlipidemic (IHL) rat models

Current study was performed in rodents by using a dual catheterization approach to support the main study. The intestine and hepatic availability of CLR at systemic and portal circulations was evaluated by a suitable model is a well-established approach and has provided opportunities to accelerate the evaluation of new drugs in rodents.<sup>24,25</sup> CLR pharmacokinetic (PK) parameters were evaluated alone and concomitant administration of number of drugs previously that, in contrast to established PK parameters, this study offers the ability to describe the CLR profiles and PK parameters in presence of HL induced by high fat diet. IHL can have a possibility to alter the liver metabolism (in disease state) henceforth IHL incidence can be evaluated. The CLR profiles and PK parameters were updated with the addition of an Atorvastatin (AT) treatment which was anti-atherosclerosis drug to better understanding of the hyperlipidemia risk. On the basis of the dual-catheterization method, CLR treated to male Wistar rats in presence and absence of hyperlipidemia with parallel design with fixed dosing. The other employing dose titration with an AT was designed based on the semi-hyperlipidemic model to further investigate CLR profiles and exposures (AUC) in male Wistar rats. In detailed objectives of the present study were twofold, First, we wanted to determine whether CLR (macrolide) metabolism and circulatory plasma concentration could affect in presence of IHL after rats fed cholesterol high fat diet and compared with early treatment or reverse the changes of TC and TG with AT which was a well-known anti-hyperlipidemic drug, second to know and confirm the down regulation of liver metabolism using Antipyrine (AP) treatment in induced hyperlipidemia and non-hyperlipidemia since AP was used as a positive control to know the liver metabolism.<sup>25</sup> Advantage of in-vivo study was physiologically and metabolically more intact system as compared with in-vitro.<sup>26</sup>

## MATERIALS AND METHODS

### Chemicals and Polyethylene tubing

Clarithromycin, Erythromycin (internal standard), Atorvastatin and Antipyrine were purchased from Sigma-Aldrich Co. (India). High performance liquid chromatography (HPLC) grade methanol and acetonitrile were obtained from Merck Co. (Darmstadt, Germany). All other chemicals in this study were of reagent grade and

used without further purification. The apparatus used in this study included a MS/MS equipped with a Agilent isocratic HPLC Pump including auto sampler (Agilent, USA), an HPLC column with temperature controller (Discovery C18, Waters), a Branson ultrasonic cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex mixer (Scientific Industries Inc., Bohemia, NY, USA), and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan). Polyethylene tubing are PE-50 ( i.d. 0.58 mm, o.d. 0.98 mm; Portex, Smiths medicals, USA) and PE-10 ( i.d. 0.28 mm, o.d. 0.61 mm; Portex, Smiths medicals, USA)

### In-vivo evaluation studies in Animals

Male Wistar rats at 10-12 weeks of age procured from in-house (Wockhardt research centre, MS, India) were housed in the laboratory with free access to a high fat diet<sup>27</sup> and normal diet (Nutrilab, provimi pvt, Ltd, India). For induction of hyperlipidemia, in-house prepared high fat diet or standard cholesterol diet was fed for 6-7 weeks separately, selected rats were fed twice daily with butter (0.5 mL) to hasten the hyperlipidemia and lipid profiles were evaluated as per the procedure published<sup>27</sup> and animals were selected based on total cholesterol (TC) levels were greater than 150 mg/dl and triglyceride (TG) levels were greater than 100 mg/dl. Diet and water maintained on a 12-h light-12-dark cycle in a room with controlled temperature and humidity. The study was conducted in accordance with the guidelines of the experimental Animal Ethics committee of the Drug metabolism and pharmacokinetics (Regd No.: 13/1999/CPCSEA, Wockhardt research centre, India). The rats were fasted for at least 12 h before the experiments.

### Preparation of model rat

After induction of successful hyperlipidemia (HL) in rats were subjected to the catheterization including non-hyperlipidemic (NHL) rats. The IHL and NHL rats were anesthetized with isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane] and surgically prepared (clipped and scrubbed). A ventral midline incision was made to expose the abdominal viscera to allow the placement of a cannula in the portal vein via the ileocolic vein and cannula was stopped advancing till 4.5 cm.<sup>28</sup> The cannula was composed of 4.5 cm of PE 10 followed by 22 cm of PE 50. The cannula was fixed firmly in place with a ligature after insertion of cannula into the vein at end part of PE 10 tubing. The end of the cannula was exteriorized subcutaneously to the dorsal base of the neck and tightened the cannula with sutures. The abdominal muscle and skin incision was closed and subsequently jugular vein cannulation was performed. Briefly, a 2 cm ventral cervical skin incision is made right of the midline with its caudal terminus at the level of the clavicle. Underlying salivary and lymphatic tissues are separated by means of blunt dissection to visualize the right common jugular vein. A sterile cannula was inserted into the vessel and secured in place with suture. Jugular vein cannula was constructed about 12 cm of polyethylene tubing. The cannula was inserted into



jugular vein about 3.5 cm towards heart. The other side of cannula tubing was passed through the back side of skin. Skin incision was closed and secured cannula port with sutures. After catheterization all cannulated rats were allowed a recovery period of 72 h followed by dosing.

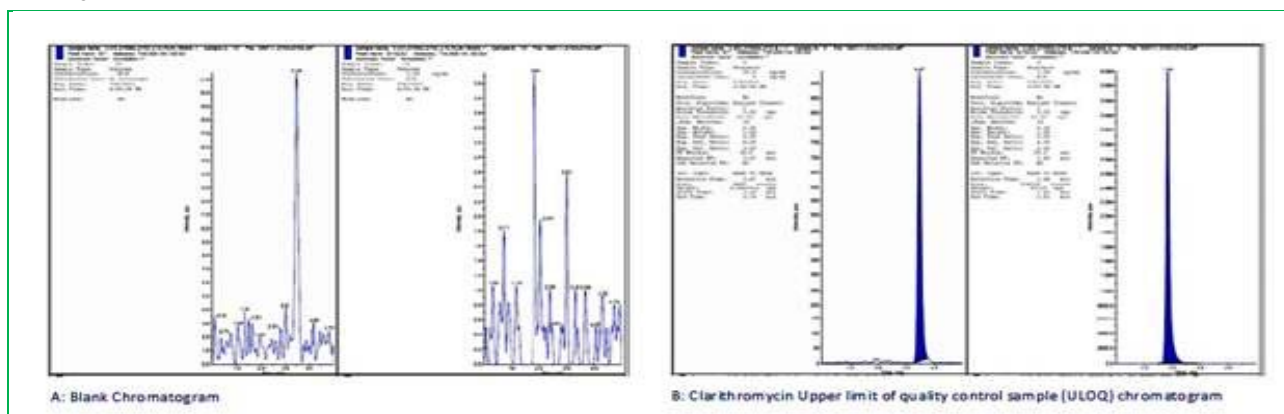
### Study design, Administration and blood sampling

The rats were divided into the following six groups (n = 6, each group) including control: CLR dose 20 mg/kg was suspended in 0.5% Carboxymethyl cellulose medium viscosity containing 2% Tween 80, (5 ml/kg) or with 0 (oral control), 20 mg/kg of AT (suspended in 0.5% Carboxymethyl cellulose in Milli-Q® water, 5.0 ml/kg) was administered intra-gastrically using an oral feeding tube, groups allocation was as scheduled, Group 1: NHL rats treated with CLR; Group 2: IHL rats treated with CLR; Group 3: IHL rats treated with concomitant administration of CLR and AT (reduced hyperlipidemic model). Group 4: Control vehicle treated. Last two groups (Group 5 and 6) AP (5 mg/kg) suspended in 0.5% carboxymethyl cellulose medium viscosity containing 2% Tween 80, (5 ml/kg) Group 5: IHL rats treated with AP; Group 6: NHL rats treated with AP. Blood samples were collected simultaneously from jugular and portal vein catheters at pre-determined time points 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post dose and 0.20 ml aliquot of blood was collected into heparinized tubes and subjected to centrifugation to obtain plasma and stored below -70 °C

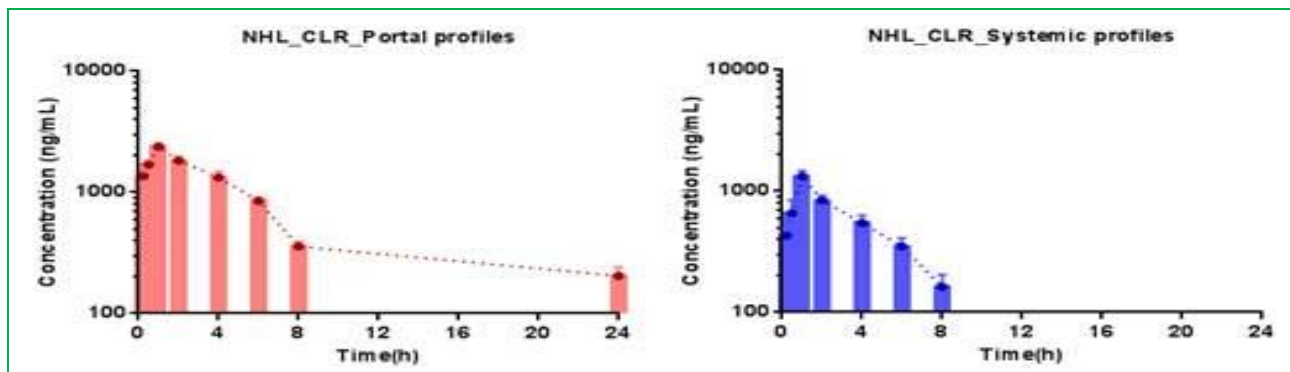
until bio analysis. For PKPD assessment first three groups were continued repeated single oral dosing up to 10 days. PK sampling was performed after single dose administration. TC and TG level were evaluated on Day 1 and Day 10 by enzymatic method to assess the CLR effect.

### Determination of the drug in the plasma

The plasma concentrations of CLR were determined using a LC-MS/MS method. Briefly, 10 µL of erythromycin, as an internal standard and a 0.2-ml aliquot of acetonitrile were mixed with a 50 µL of plasma sample. The resulting mixture was then vortex mixed for 2 min and centrifuged at 10,000 rpm for 10 min. A 100 µL aliquot of the supernatant was injected (Injection volume, 10 µL) into the LC-MS/MS system. The chromatographic separations were achieved using waters, discovery C18 column (4.6 x 50 mm, 5 µm). The mobile phase consisted of 30% of 5 mM ammonium formate and 70% of acetonitrile containing 0.1% formic acid. The flow rate of the mobile phase was maintained at 0.5 mL/min. The respective chromatograms of ULOQ, matrix blank, and LLOQ samples are shown in Figures 1. The clean chromatogram of matrix blank obtained from the injection of a matrix blank extract immediately after an ULOQ sample demonstrated that this method had neither injector carryover nor analytical column carryover. Calibration curve, accuracy, precision and recovery data was not showed as was sent for publication.



**Figure 1:** Clarithromycin LC-MS/MS chromatograms: A) Blank and B) ULOQ with Erythromycin as an internal standard



**Figure 2:** Mean portal and systemic plasma concentration-time profiles of CLR after oral administration of CLR (20 mg/kg) formulation to rats in the absence of hyperlipidemia (NHL). The bars represent the standard deviation. Filled pink columns bars represent the CLR mean concentrations (n=6) in portal after oral administration. Filled blue columns bars represent the CLR mean concentrations (n=6) in systemic after oral administration.

**Table 1:** Mean pharmacokinetic parameters of Clarithromycin after single oral administration of CLR alone (20 mg/kg) and concomitant administration of AT (20 mg/kg; n=6, mean; SD) to cannulated rats in the absence or presence of induced hyperlipidemia

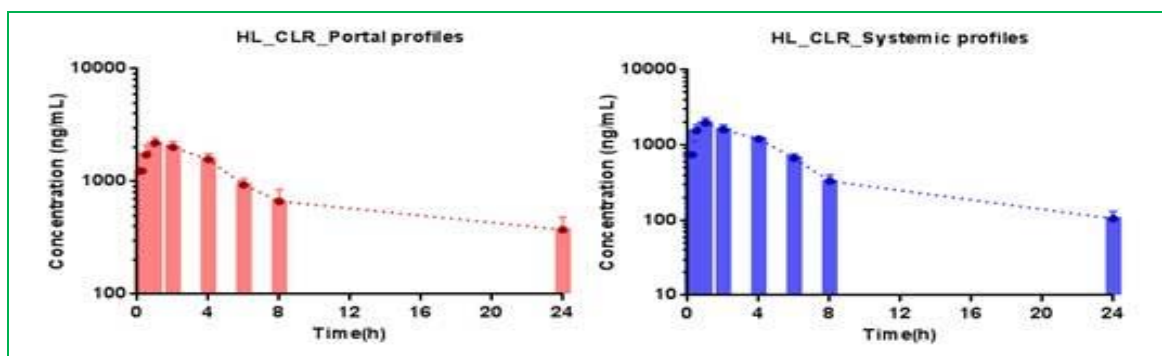
| PK parameters                     | NHL_CLR      | HL_CLR        | HL_CLR_AT    |
|-----------------------------------|--------------|---------------|--------------|
|                                   | Mean ± SD    | Mean ± SD     | Mean ± SD    |
| T <sub>max</sub> ; por (h)        | 1.00         | 1.08 ± 0.49   | 1.50 ± 0.55  |
| T <sub>max</sub> ; sys (h)        | 1.00         | 0.92 ± 0.20   | 0.75 ± 0.27  |
| C <sub>max</sub> ; por (µg/mL)    | 2.40 ± 0.2   | 2.20 ± 0.25   | 2.13 ± 0.3   |
| C <sub>max</sub> ; sys (µg/mL)    | 1.33 ± 0.15  | 1.98 ± 0.30*  | 1.69 ± 0.3   |
| AUC <sub>last por</sub> (µg.h/mL) | 14.73 ± 1.05 | 21.30 ± 1.75* | 15.55 ± 1.28 |
| AUC <sub>last sys</sub> (µg.h/mL) | 5.86 ± 0.81  | 12.25 ± 0.56* | 7.98 ± 0.73  |

SD: standard deviation; por: Portal; sys: Systemic; \* P<0.01, significantly different as compared with control (NHL \_ CLR) using student's t-test

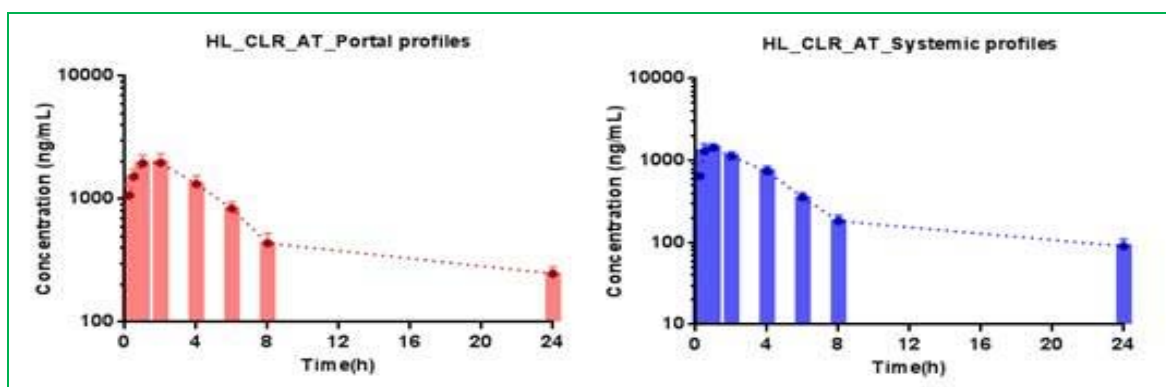
**Table 2:** Estimated the intestine and liver availability of Clarithromycin after oral administration of Clarithromycin alone and concomitant administration of Atorvastatin to cannulated rats in the presence or absence of hyperlipidemia (Dose: 20 mg/kg for CLR and AT; n=6)

| Groups    | F <sub>a</sub> .F <sub>g</sub> | F <sub>h</sub> | E <sub>h</sub> | F    | Q portal |
|-----------|--------------------------------|----------------|----------------|------|----------|
| NHL_CLR   | 0.88                           | 0.40           | 0.60           | 0.35 | 32.9     |
| HL_CLR    | 0.89                           | 0.58           | 0.42           | 0.51 |          |
| HL_CLR_AT | 0.75                           | 0.51           | 0.49           | 0.38 |          |

Q portal: portal blood flow; CLR: Clarithromycin; AT: Atorvastatin



**Figure 3:** Mean portal and systemic plasma concentration-time profiles of CLR after oral administration of CLR (20 mg/kg) formulation to rats in the presence of IHL. The bars represent the standard deviation. Filled pink columns bars represent the CLR mean concentrations (n=6) in portal after oral administration. Filled blue columns bars represent the CLR mean concentrations (n=6) in systemic after oral administration.



**Figure 4:** Mean portal and systemic plasma concentration-time profiles of CLR after oral co-administration of CLR (20 mg/kg) and AT (20 mg/kg) to rats in the presence of induced hyperlipidemia (HL). The bars represent standard deviation. Filled pink columns represent the CLR mean concentration (n=6) in portal after oral co-administration of AT and CLR formulations. Filled blue columns represent the CLR mean concentrations (n=6) in systemic after oral co-administration of AT and CLR formulations.

## Data Analysis

The plasma concentration data was analysed using a non-compartmental method of WinNonlin software version 2.1 (Pharsight Co., Mountain View, CA, USA). The maximum plasma concentration ( $C_{max}$ ) and time to reach the peak concentration ( $T_{max}$ ) of Clarithromycin in the plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration–time curve ( $AUC_{last\ or\ 0-t}$ ) from time zero to the time of the last measured concentration ( $C_{last}$ ) was calculated using the linear trapezoidal rule.

The apparent intestinal absorption coefficient ( $F_a.F_g$ ) and liver extraction ratio was calculated as follows:

$$F_a.F_g = Q_{por} \times R_b (AUC_{por} - AUC_{sys}) / Dose\ oral \quad (1)$$

Where  $F_a$ ,  $F_g$  and  $Q_{por}$  are fraction absorbed at gut, and portal vein blood flow, respectively.  $Q_{por}$  value for rat was assumed to be 32.9 mL/min/kg.<sup>25</sup>  $AUC_{por}$ : Following oral dose, sampling from Portal vein Area Under the curve;  $AUC_{sys}$ : Following oral dose, sampling from Jugular vein Area Under the curve

$$E_h = AUC_{por} - AUC_{sys} / AUC_{por} \dots \dots \dots (2)$$

$$F_h = 1 - E_h \dots \dots \dots (3)$$

## Statistical analysis

Statistical analysis was carried out using a student's paired t-Test with a two tailed distribution. The differences were considered significant at a level of  $P < 0.01$  for all groups. All mean values are presented with their standard deviation (Mean  $\pm$  SD).

## PKPD assessments

The same serial sampling approach was utilized to collect blood samples and an aliquot of the PK samples were transferred into PD sample vial at pre-determined time points are pre-dose and 0.25, 1, 4, 8 and 24 h post-dose for evaluation of TC and TG levels in all groups. Thereafter dosing was continued up to 10 days for group 1, group 2 and group 3. On day 10, post-dose blood samples were collected at the predetermined samples as stated above and TC and TG levels were estimated. The serum cholesterol and triglycerides levels (mg/dl) were estimated by enzymatic method<sup>29</sup> and compared Day 01 results with Day 10 to investigate any changes of TC and TG levels after 10 day repeated dosing of CLR.

## RESULTS

Evaluation of CL pharmacokinetic parameters in absence and presence of hyperlipidemia induced by high fat diet in male Wistar rats following oral administration of CLR alone and concomitant administration of AT.

CLR availability at intestine (portal) and liver (systemic) in NHL Wistar rats

The portal and systemic plasma concentration profiles of CLR in NHL rats at predetermined time points are shown

in Figure 2. Non-compartmental model estimation was used to report pharmacokinetic parameters which were parameterized in terms of  $C_{max}$ ,  $T_{max}$ , and  $AUC_{last}$  mean results are displayed in Table 1. The portal  $C_{max}$  and  $AUC_{last}$  exposures were 2.4  $\mu\text{g/mL}$  and 14.73  $\mu\text{g/mL} \times \text{h}$ , respectively and similarly systemic  $C_{max}$  and  $AUC_{last}$  exposures were 1.33  $\mu\text{g/mL}$  and 5.86  $\mu\text{g/mL} \times \text{h}$ , respectively. Portal  $AUC_{last}$  was 2.5-fold and  $C_{max}$  was 1.8-fold greater than systemic exposures which reflects significantly ( $P < 0.01$ ) lower concentrations at systemic side as compared with portal. The  $F_a.F_g$  was found to be 0.88 suggests almost of all drug enters in to portal circulation in absence of hyperlipidemia. The fraction of Clarithromycin escaping from the liver ( $F_h$ ) was 0.40 and % bioavailability was found to be 35 in Wistar rats and results are summarized in Table 2.

## Clarithromycin availability at intestine and liver in induced hyperlipidemic Wistar rats

The portal and systemic plasma concentration profiles of CLR in IHL rats at predetermined time points are shown in Figure 3. Non-compartment model estimation was used to report pharmacokinetic parameters which were parameterized in terms of  $C_{max}$ ,  $T_{max}$ , and  $AUC_{last}$  mean results are displayed in Table 1. The portal  $C_{max}$  and  $AUC_{last}$  exposures were 2.2  $\mu\text{g/mL}$  and 21.30  $\mu\text{g/mL} \times \text{h}$ , respectively and similarly systemic  $C_{max}$  and  $AUC_{last}$  exposures were 1.98  $\mu\text{g/mL}$  and 12.25  $\mu\text{g/mL} \times \text{h}$ , respectively. Portal  $AUC_{last}$  was 1.74-fold and  $C_{max}$  was 1.1-fold greater than systemic exposures. Induced hyperlipidemic rat systemic concentrations were significantly ( $P < 0.01$ ) high as compared with non-hyperlipidemic rat systemic concentrations. The apparent intestinal absorption coefficient ( $F_a.F_g$ ) was found to be 0.89 and the fraction of CLR escaping from the liver ( $F_h$ ) was 0.58 strongly suggests and confirmed with our previous results (data was not shown) that is the liver metabolism was reduced in presence of hyperlipidemia and % bioavailability (F) was found to be 51 proved that F was significantly high in hyperlipidemia as compared with non-hyperlipidemia. Results are shown in Table 2. The same outcome was already we studied in single dose PK and biliary excretion of Clarithromycin (data was not shown as was shared for publication).

## CLR availability at intestine and liver in IHL Wistar rats with concomitant administration of Atorvastatin

The portal and systemic plasma concentration profiles of CLR at predetermined time points are shown in Figure 4. Non-compartment model estimation was used to report pharmacokinetic parameters which were parameterized in terms of  $C_{max}$ ,  $T_{max}$ , and  $AUC_{last}$  mean results are displayed in Table 1. The portal  $C_{max}$  and  $AUC_{last}$  exposures were 2.13  $\mu\text{g/mL}$  and 15.55  $\mu\text{g/mL} \times \text{h}$ , respectively and similarly systemic  $C_{max}$  and  $AUC_{last}$  exposures were 1.69  $\mu\text{g/mL}$  and 7.98  $\mu\text{g/mL} \times \text{h}$ , respectively. Portal  $AUC_{last}$  was 1.94-fold and  $C_{max}$  was 1.26-fold higher than systemic exposures. CLR systemic concentrations in IHL rats treated with AT (Dose: 20 mg/kg; Group 3)) were



significantly ( $P < 0.01$ ) low as compared with IHL rat CLR systemic concentrations (Group 2). The  $F_a, F_g$  was found to be 0.75 and the  $F_h$  was 0.51 demonstrated that the liver metabolism was recovered in presence of AT in hyperlipidemia conditions and which is almost similar as compared with NHL rats. Evaluated and proved that HL was a major concern which was altered CLR metabolism. The % bioavailability was found to be 38 in Wistar rats confirmed that induced hyperlipidemia alters the systemic bioavailability. The bioavailability has been reduced after AT administration since AT was an anti-hyperlipidemic drug which reduced lipid profiles to normal as compared with NHL. Results are summarized in Table 2.

### AP availability at intestine and liver in Wistar rats in absence and presence of IHL

The portal and systemic plasma concentration profiles of AP in absence and presence of induced hyperlipidemic rats at predetermined time points are shown in Figure 5 and 6. Non-compartment model estimation was used to report pharmacokinetic parameters which were parameterized in terms of  $C_{max}$ ,  $T_{max}$ , and mean  $AUC_{last}$ . In absence of hyperlipidemia, the portal  $C_{max}$  and  $AUC_{last}$  exposures were 12.7 mg/mL and 53 mg/mL x h, respectively and similarly systemic  $C_{max}$  and  $AUC_{last}$  exposures were 13.5 mg/mL and 42 mg/mL x h, respectively. Portal  $AUC_{last}$  was 21% greater than systemic exposure.

**Table 3:** Hepatic extraction ratio and fraction of drug escaping from liver of AP after oral administration of AP formulation to cannulated rats in the presence or absence of IHL (Dose: 20 mg/kg for AP; n=6)

| PK parameters                | Mean $\pm$ SD (PO) |                  |
|------------------------------|--------------------|------------------|
|                              | NHL_AP             | HL_AP            |
| $T_{max}$ ; portal (h)       | 0.25               | 0.25             |
| $T_{max}$ ; systemic (h)     | 0.25               | 0.25             |
| $C_{max}$ ; portal (mg/mL)   | 12.67 $\pm$ 1.08   | 10.98 $\pm$ 0.8  |
| $C_{max}$ ; systemic (mg/mL) | 13.5 $\pm$ 0.85    | 13.98 $\pm$ 0.96 |
| $AUC_{portal}$ (mg.h/mL)     | 53 $\pm$ 14.1      | 59 $\pm$ 15      |
| $AUC_{systemic}$ (mg.h/mL)   | 42 $\pm$ 13.8      | 54 $\pm$ 5.73    |
| $E_h$                        | 0.21               | 0.08             |
| $F_h$                        | 0.79               | 0.92             |

SD: standard deviation.  $F_a, F_g$  was considered as 1 (as per the literature value)

In presence of hyperlipidemia, the portal  $C_{max}$  and  $AUC_{last}$  exposures were 10.98 mg/mL and 59 mg/mL x h, respectively and similarly systemic  $C_{max}$  and  $AUC_{last}$  exposures were 13.98 mg/mL and 54 mg/mL x h, respectively. But in IHL rat the exposure differences was 8% increased as compared with absence of hyperlipidemic rats. IHL rat systemic concentrations were significantly ( $P < 0.01$ ) greater than non-hyperlipidemic rat systemic concentrations. The  $F_a, F_g$  was considered as 1 (Masuta et al, 2012) and the fraction of AP escaping from

the liver ( $F_h$ ) was 0.79 and 0.92 for absence and presence of induced hyperlipidemia, respectively. Similarly liver extraction ratios were 0.21 and 0.08 for absence and presence of induced hyperlipidemic rat models, respectively. Liver metabolism was reduced in presence of induced hyperlipidemia as compared with absence of hyperlipidemia. Results are shown in Table 3.

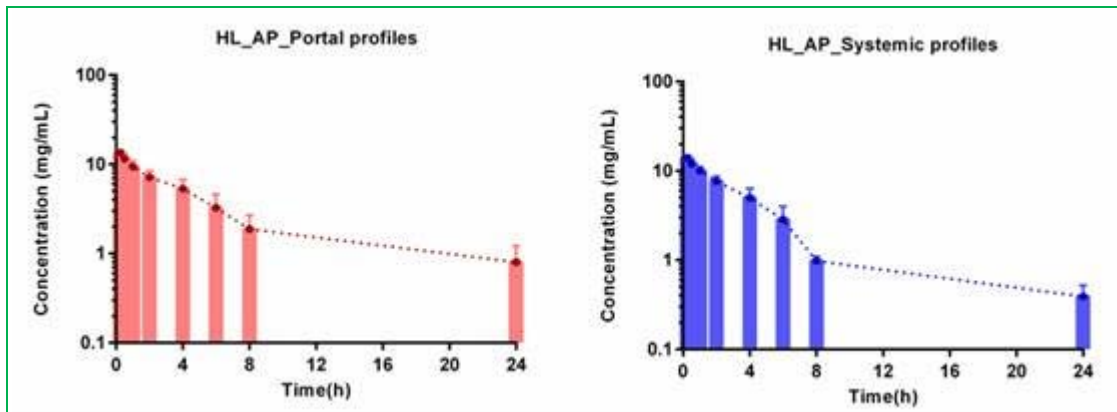
### PKPD assessments

The induced hyperlipidemia was confirmed after estimation of TC and TG by enzymatic method. Induced hyperlipidemic rats had severely high in total cholesterol (>150 mg/dl) and triglyceride levels (>100 mg/dl) as compared with mean baseline fasting cholesterol and triglycerides levels. The selected induced hyperlipidemic rats and non-hyperlipidemic rats were allocated for PK and PD study design. On Day 1 post dose plasma samples were collected and estimated TC and TG levels thereafter continued dosing up to 10 days. On day 10 post dose plasma samples were collected and estimated for TC and TG levels. The pictorial characteristics of virtual cholesterol levels and triglyceride levels in male Wistar rats are presented in Figure 7. TC and TG levels were estimated and compared (day 1 and day 10) with base line levels. In non-hyperlipidemic rat model, The TC levels on Day 1 are comparable with the base line but 10% increase on Day 10 was observed. Similarly, in induced hyperlipidemic rat models observed 10% increase in TC on day 10 as compared with day 1 and base line levels. In reduced hyperlipidemic rat model a 27% reduction was found in TC on day 10 after co-administration of Atorvastatin as compared with day 1 and base line levels. The estimated mean change from baseline values for TC and TG in absence and presence of hyperlipidemia on Day 1 and Day 10 after CLR alone and concomitant administration of Atorvastatin.

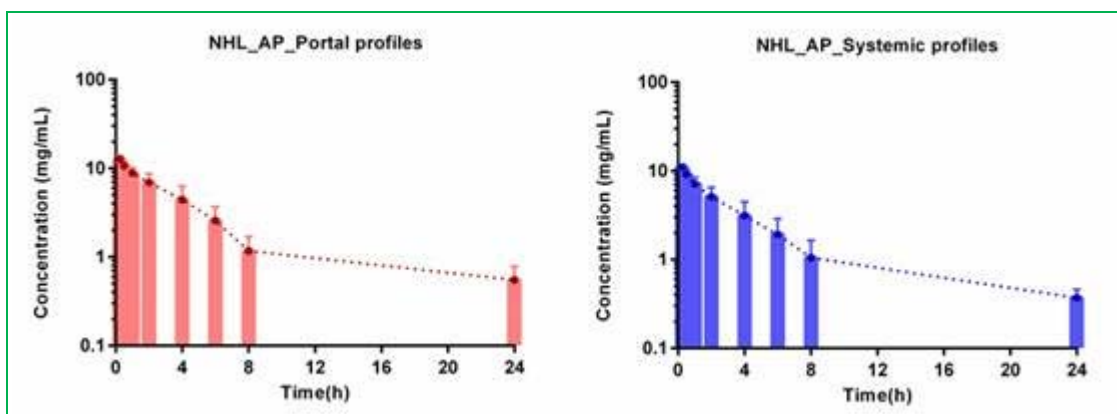
The TC results suggests that there would be a mean change in TC of 84 mg/dl to 92 mg/dl from day 1 to day 10 in absence of hyperlipidemia and 186 mg/dl to 205 mg/dl from day 1 to 10 in presence of hyperlipidemia, respectively with a fixed dosing of CLR. As expected, the incidence of hypercholesteromic effort was found to be increased from day 1 to 10 following CLR repeated single dosing in absence and presence of hyperlipidemia. In addition, TG levels were also estimated from the same collected sample. The observed plasma TG levels on day 1 and day 10 in absence and presence of hyperlipidemia after CLR treatment and compared. Results suggests that there would not be a mean change in TG in absence of hyperlipidemia but a significant difference was observed on day 1 and day 10 in presence of induced hyperlipidemic rats about 18% was increased as compared with baseline levels. There also appears to be reasonable agreement between day 1 and day 10 in the induced hyperlipidemia incidence. Well illustrated in Figure 7. The CLR repeated treatment in induced hyperlipidemic rat was demonstrated approximately 10% increases in TC and 17% increase in TG levels on day 10 as compared with baseline levels and on day 1. Similarly, in

absence of hyperlipidemia, only 10% increase in TC and no significant change in TG levels were observed. But in reduced hyperlipidemic rat model, TC and TG were significantly reduced on day 10 (130 mg/dl for TC; 106 mg/dl for TG) as compared with the baseline levels (185

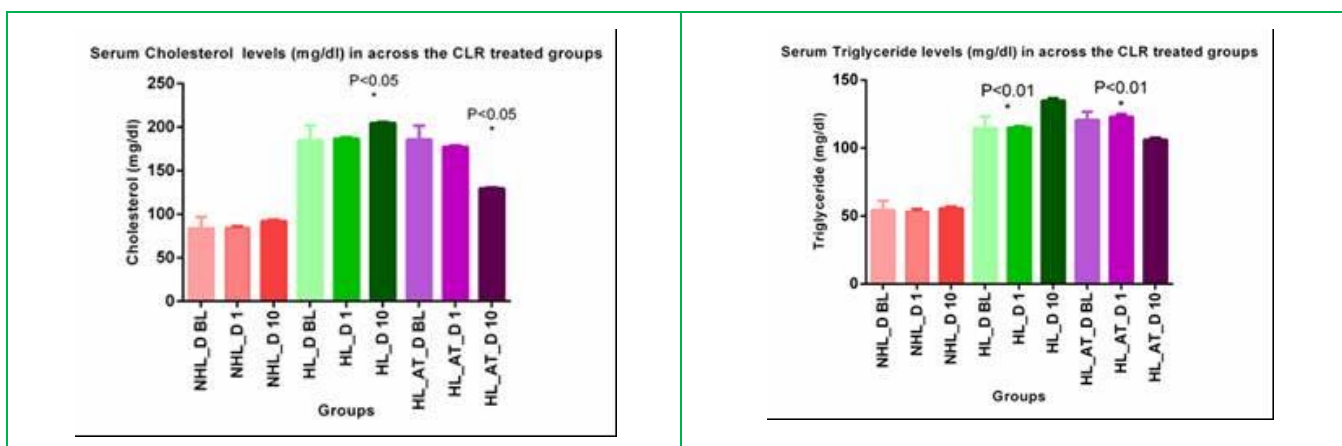
mg/dl for TC; 121 mg/dl for TG) and on day 1 (179 mg/dl for TC; 123 mg/dl for TG). After correction for the hyperlipidemia by administrating the anti-hyperlipidemic drug like AT, the TC and TG decrease is approximately 27% and 14%, respectively.



**Figure 5:** Mean portal and systemic plasma concentration-time profiles of AP after oral administration of AP (20 mg/kg) formulation to rats in the NHL. The bars represent the standard deviation. Filled pink columns represent the AP mean concentrations (n=6) in portal after oral administration, filled blue columns represent the AP mean concentrations (n=6) in systemic after oral administration.



**Figure 6:** Mean portal and systemic plasma concentration-time profiles of AP after oral administration of AP (20 mg/kg) formulation to rats in the presence of IHL. The bars represent the standard deviation. Filled pink columns represent the AP mean concentrations (n=6) in portal after oral administration, filled blue columns represent the AP mean concentrations (n=6) in systemic after oral administration.



**Figure 7:** Mean TC levels (mg/dl) and TG (mg/dl) levels on day 1 and day 10 compared with base line levels after repeated dose administration of CLR alone and co-administration of AT with fixed dose of 20 mg/kg to male Wistar rats in the NHL or HL. The bars represent the standard deviation. Filled pink columns NHL\_D BL represent the base line levels prior to the dosing. Filled pink columns NHL\_D 1 represents TC and TG levels on day 1 after single dose administration. Filled pink columns NHL\_D 10 represent the TC and TG levels on day 10 after repeated dose administration. Filled green columns HL\_D BL represent the base line TC and TG levels prior to the

dosing. Filled green columns HL\_D 1 represent the TC and TG levels on day 1 after single dose administration. Filled green columns HL\_D 10 represent the TC and TG levels on day 10 after repeated dose administration. Filled violet columns HL\_AT\_D BL represent the base line TC and TG levels prior to the dosing. Filled violet columns HL\_AT\_D 1 represent the cholesterol levels on day 1 after single dose administration of CLR and AT. Filled violet columns HL\_AT\_D 10 represent the TC and TG levels on day 10 after repeated dose administration of CLR and AT. Pink: CLR treated in absence of hyperlipidemia; Green: CLR treated in presence of hyperlipidemia; Violet: CLR and AT co-administered in reduced hyperlipidemic conditions. NHL: absence of hyperlipidemia; HL: presence of induced hyperlipidemia.

## DISCUSSION

The main objective of this work was to evaluate the CLR profiles and availability at intestine and liver using hyperlipidemic, semi-hyperlipidemic and non-hyperlipidemic rat models and to investigate the impact of induced hyperlipidemia on CLR availability and PK parameters. The impact of HL on CLR availability was evaluated and also investigated CLR availability in presence of AT. IHL would only enhance the availability of CLR at liver by reducing its metabolism. However metabolism at intestinal level would not alter CLR levels significantly. We demonstrated that availability of CLR in portal circulation ( $F_a.F_g$ ) was not altered but hepatic metabolism of CLR was significantly altered (down regulation) in presence of induced hyperlipidemia as compared with absence (non-hyperlipidemia).

Many reports in the biomedical literature have documented that hepatic disease can alter the absorption and disposition of drugs (PK) as well as their efficacy and safety (PD). These reports have been based on studies in patients with common hepatic diseases. The specific impact of any disease on hepatic function is often poorly described and highly variable, particularly with regard to effects on the PK and PD of a drug.<sup>14</sup>

In current study,  $F_a.F_g$  and  $F_h$  are estimated following a single oral administration of compound in rats with portal and jugular vein catheterization which helps to withdraw the blood samples to estimate local AUC of portal and systemic circulation, respectively. An additional advantage of this dual catheterization model is that  $F_a.F_g$  and  $F_h$  can be calculated from a single oral dose administration studies and estimation of non-hepatic clearance was not required. Various in vivo and in vitro methods are used to assess oral F and the extent of first-pass metabolism in the liver and intestine.<sup>30-32</sup> Macrolide antibiotics Azithromycin and CLR are large molecular weight compounds and are substrates for apically polarized efflux transporters such as P-glycoprotein, which can potentially restrict intestinal absorption. However, despite these undesired physicochemical and biopharmaceutical properties, CLR exhibited moderate to excellent p.o. bioavailability in preclinical species and humans. Intestinal uptake transporters, such as organic anion transporting polypeptides (OATPs), can facilitate the uptake of drugs that are substrates and hence increase p.o. absorption.<sup>33</sup> CLR interactions in ex-vivo were already proved under normal conditions but in presence of induced hyperlipidemia CLR exposure was not studied. We evaluated CLR exposures or area under the curves (AUC) in presence of induced hyperlipidemic rat modes, in addition, also investigated after co-

administration of AT with CLR to confirm the influence of IHL.

Disorders of lipid metabolism have received limited research but recent scientific evidence suggests that it can often be associated with diseases such as pancreatitis, hepatobiliary disease, ocular disease, and seizures. Therefore, hyper triglyceridemia should always be taken into account and appropriately treated when necessary.<sup>34</sup> The clearance (non-renal clearance) of CLR in the DMIS rats became significantly slower (by 21.6%) than that in the DMIA rats although the exact reason for this was unclear.<sup>35</sup> Lee et al, 2012, investigated CLR hepatic clearance was lower in presence of diabetics similarly we demonstrated that CLR systemic availability was significantly high (low clearance) in hyperlipidemic rats as compared with non-hyperlipidemia (Table 1). Alanine amino transaminase (ALT) in the serum may be increased with acute damage to hepatic cells.<sup>36</sup> The abnormal values of ALT may cause significant changes in pharmacokinetic profiles of any tested drug.<sup>36</sup> Therefore, we investigated the influence of induced hyperlipidemia and AT on CLR pharmacokinetics, proved that CLR pharmacokinetic parameters have been changed in presence of induced hyperlipidemia as compared with absence of hyperlipidemia.

To examine potential kinetic interactions between HL and CLR and then explored whether HL could increase  $AUC_{last}$  by inhibition of hepatic metabolism. Portal and jugular vein dual cannulation was performed to check the CLR availability at intestine and hepatic levels. The intestinal CLR escaping from the gut ( $F_a.F_g$ ) and fraction of CLR escaping from liver ( $F_h$ ) was determined in non-hyperlipidemic Wistar rats, found that  $F_a.F_g$  was 0.88 and  $F_h$  was 0.40 suggests that there was no significant gut metabolism but liver metabolism was found to be about 50%. The oral absolute bioavailability was found to be 35% in non-hyperlipidemic rats, In addition to our investigations, intestinal uptake and almost similar bioavailability was reported in non-hyperlipidemia conditions in earlier studies<sup>33</sup> but no investigations have been published on CLR pharmacokinetic parameters in presence of IHL so rat pharmacokinetic studies were conducted to explore whether the attenuated exposure of Clarithromycin in the presence of hyperlipidemia. Hyperlipidemic rats showed there was no significant intestinal metabolism,  $F_a.F_g$  found to be 0.89 but significantly greater ( $p < 0.01$ )  $F_h$  was found compared to absence of hyperlipidemia which was 0.58 indicates that hyperlipidemia has a significant impact on hepatic metabolism and facilitates more hepatobiliary excretion (Data was not shown).





Liver metabolism was reduced to almost 30% as compared to non-hyperlipidemic rats. Finally oral bioavailability significantly high and was found to be 51%. Although CLR uptake by hepatic OATP1B1 and OATP1B3 has been shown, the interaction of intestinal OATPs has been investigated<sup>37</sup> but no study has been conducted in hyperlipidemia conditions to know the Clarithromycin availability at intestine ( $F_a$ ,  $F_g$ ) and liver ( $F_h$ ) in awakening rats. The result of our study suggests that hyperlipidemia has a significant impact on availability of CLR at liver ( $F_h$ ) and overall oral bioavailability ( $F$ ). To assess the role of hyperlipidemia, we had run an additional parallel group treated by AT (single oral administration; 20 mg/kg) found that slightly  $F_h$  was reduced to 0.51 which was almost similar to non-hyperlipidemic rats and no significant change in intestinal availability. Since induced hyperlipidemia causes the down regulation of liver metabolism the exact mode of action was not known might be altering the apolipoproteins levels and CYP mediated metabolism could be the one of the reason. AP was used as a positive control to know the liver metabolism in induced hyperlipidemic rat model and non-hyperlipidemic rat model and compared. The key objective for CLR was to identify impact of induced hyperlipidemia on CLR pharmacokinetics that provide greater increase in the CLR exposure (AUC) and/or significantly increased the percentage dose excreted in the bile as compared to non-hyperlipidemia (data was not shown). Similar measures of hepatic function have been sought using endogenous substances affected by the liver such as bilirubin and albumin, or functional measures such as prothrombin time, or the ability of the liver to eliminate marker substrates such as AP,<sup>38</sup> indocyanine green (ICG).<sup>38</sup> To have a valid support, we were evaluated liver metabolism using antipyrine as a marker.

### Antipyrine assessment

Antipyrine has a low hepatic extraction ratio.<sup>38</sup> It is almost completely oxidized by various hepatic enzymes (2-hydroxylation and 1-N-demethylation) and is limited by metabolic capacity of the intrinsic enzyme activity and not necessarily by hepatic blood flow or hepatic uptake. It has been used extensively as a general marker for the functional ability of the cytochrome P-450 oxidative pathway and is affected by a wide range of liver diseases (e.g., chronic liver disease, hepatitis, and cirrhosis). The liver extraction ratio and fraction of drug escaping from liver values of AP were evaluated in absence and presence of IHL rat models. The model was proved a 2.5-fold lower hepatic extraction ratio in presence of IHL as compared with absence of hyperlipidemia. The IHL model well demonstrated to estimate the hepatic extraction ratio and appears to be reasonable agreement between CLR and AP data (Table 3)

### PKPD assessments

The totals of 11,583 Clarithromycin users are studied to evaluate hyperlipidemia reported to have a side effects when taking CLR among them 105 people have

hyperlipidemia.<sup>14</sup> The evidence is now strong for a link between lipid abnormalities, cardiovascular disease and CLR. HL is an increase in the amount of fat (such as TC and TG) in the blood. The TC and TG were considered as primary parameters since these parameters will have effect initially in HL. We investigated TC and TG levels in absence and presence of hyperlipidemia to assess the PKPD co-relations. The result of TC and TG has been suggested to potentially revolutionize the conduct of this experiment in presence and absence of hyperlipidemia. Investigation of detailed mode of action is required. C. pneumonia infection has also been demonstrated to enhance the aortic changes seen with a cholesterol-enriched diet in the rabbit and to accelerate atherosclerosis in hypercholesterolemic murine models.<sup>13</sup> In successfully induced hypercholesterolemic models has been exhibited a high mean total concentrations of cholesterol are 57.7 versus 56.8 mmol/liter in two groups<sup>13</sup> and literature review was supported TC and TG levels were enough to monitor the hyperlipidemia.

### CONCLUSION

In conclusion, the CLR metabolism on hyperlipidemic disease model approach experiencing catheterizations outcome according to the metabolism at liver of a drug is promising. The estimated higher TC and TG level during the CLR treatment in presence of IHL and increased hyperlipidemia risk provided the basis for the decision to focus on CLR metabolism and for the selection of doses for the demonstration of superiority of CLR systemic availability in presence of IHL as compared to NHL; and was largely confirmed by the pre-clinical rat results. The high cholesterol diet induced rat model represents a unique tool for the evaluation of CLR systemic availability, hepatic extraction ratio and fraction of drug availability at intestine and liver in presence of hyperlipidemia incidence, and was used to guide further investigation. The pharmacodynamic exercise yielded valuable insight into how PKPD-based co-relations of pre-clinical study make an impact on decision making in drug development.

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