Comparative Pharmacokinetics of Rosuvastatin in Male C57BL/6 Mice Following Single Intravenous and Oral Administration co-Administered With Herbal Bioenhancers

Sudipta Basu1,*, Vandana B. Patel2

1Sai Advantium Pharma Ltd; Department of DMPK and Toxicology, International Biotech Park, Phase 2, Hinjewadi, Pune- 411057, Maharashtra, India.
2Babaria Institute of Pharmacy, Vadodara - Mumbai NH # 8, Varnama, Vadodara-391240, Gujarat, India.

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ABSTRACT

Bioavailability is the rate and extent to which a therapeutically active substance enters systemic circulation and becomes available at the required site of action [1]. Intravenous drugs attain maximum bioavailability, while oral administration yields a reduced percentage due to incomplete drug absorption and first-pass metabolism. Methods of increasing bioavailability of a drug correspondingly increase levels in the bloodstream, and thus the efficacy, which in turn reduces the drug dosage required to achieve a given therapeutic effect. Until now, methods of increasing drug bioavailability have operated within a narrow manipulative framework, mainly based on physical processes including micronization, deaggregation of micronized molecules, timed/site release preparations, solubilization of active drug and polymorphic/crystal form selection and nanotechnology (nanotechnology is at the experimental stage so it is a promising future method).

Rosuvastatin (RSV), a 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitor, has been developed for the treatment of patients with primary hypercholesterolemia and mixed dyslipidemia [2, 3]. Rosuvastatin predominantly undergoes biliary excretion, and 80-90% of a single p.o. administered dose is recovered unchanged in feces, indicating that carrier-mediated hepatic transport may be important for disposition and potential drug interactions with rosuvastatin [4-7]. Members of the organic anion transporting polypeptide (OATP) superfamily have been shown to be active transporters of rosuvastatin [4-6]. Co-administered of rosuvastatin with gemfibrozil and cyclosporine, which are known inhibitors of OATP1B1, led to an increase of rosuvastatin Cmax (2–11-fold) and area under the curve (AUC) (2–7-fold) [6, 7].

Bioenhancers are drug facilitators they are the molecules which by themselves do not show typical drug activity, but when used in combination enhance the activity of drug molecules in several ways. Moreover, efficacy is enhanced by increased bioavailability [8, 9]. The exact mechanism of action for increasing bioavailability is unknown, it might enhance the gastrointestinal (GI) absorption, might inhibit GI wall metabolism [10], might enhance the reabsorption from the renal tubules, might increase the unionized fraction at the receptor sites or might be acting as an inhibitor of transporters [11].

There is an increasing interest and medical need for the improvement of bioavailability of a large number of drugs. The promising approaches, the co-administration of therapeutic agents with natural compounds possessing absorption improving activities has gained great interest in oral drug delivery [12].

Bioavailability differs greatly from one polyphenol to another [13]. Effect of two new herbal compounds viz. gallic acid and cinnamic acid as a bioenhancer, several articles are available [14-16]. Polyphenols are abundant bioavailability is unknown it might enhance the gastrointestinal (GI) absorption, might inhibit GI wall metabolism [10, 12]. The least well-absorbed bioavailability of drugs like vascicine, sparteine, curcumin, barbiturate and oxyphenylbutazone, zoxazolamine, propranolol and theophylline in animal experiments [13-15]. Polyphenols are abundant microinutrients in our diet, and evidence for their role in the prevention of many diseases [16]. Bioavailability differs greatly from one polyphenol to another [17]. Many natural compounds from medicinal plants have demonstrated capacity to enhance the bioavailability when co-administered. These natural compounds include quercetin, genistein, naringenin, sinomenine, piperine, glycyrrhizin and nitrile glycoside [18]. Apart from that there are other herbal compounds, having capacity to enhance bioavailability [19]. Plant foods contain a variety of medicinally active constituents such as alkaloids (piperine) and polyphenols (cinnamic acid and gallic acid), which are increasingly regarded as herbal medicines [20]. Piperine has also been shown to enhance the bioavailability of drugs like vascicine, sparteine, curcumin, barbiturate and oxyphenylbutazone, zoxazolamine, propranolol and theophylline in animal experiments [14-16]. Polyphenols are abundant microinutrients in our diet, and evidence for their role in the prevention of many diseases [16]. Bioavailability differs greatly from one polyphenol to another [17].

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MATERIALS AND METHODS

Chemicals and Reagents
Rosuvastatin calcium (cat. no. # 23551) was procured from Aapin Chemicals (Abingdon, Oxon, UK). Atorvastatin calcium (cat. no. # P20001), piperine (lot no. # U13423-458), gallic acid (lot no.# 53540), cinnamic acid (lot no. # W228826), ammonium acetate buffer (lot no. # BCBB1360V), propylene glycol (lot no. #1359720) and formic acid (lot no. # BCBC4518V) were procured from Sigma-Aldrich (Aldrich, St. Louis, MO). Acetonitrile and methanol were of HPLC grade procured from JT Baker (Phillipsburg, NJ, USA). Demineralized water was prepared in-house using milli-pore water system (USA). Absolute ethanol (99.9%) was purchased from Tedia Company, Inc (Fairfield, OH, USA). All the other reagents or solvents used were either analytical or high-performance liquid chromatography (HPLC) grade.

Animals
A total forty five male C57BL/6 mice (25-30 g) were obtained from Relevance Life Sciences Pvt. Ltd. (Mumbai, India). The mice were 10-12 weeks old at the time of testing. All animals were quarantined for 7 days and examined to assure that they were healthy before release from quarantine. Mice were randomly assigned five groups containing 9 mice in each group. Randomization was based on a modified Latin square. Animals were housed in the sterilized polycarbonate cages in an environmentally controlled room. Temperature and humidity was maintained at 22 ± 3°C and 40-70%, respectively and illumination was controlled to give a sequence of 12 h light and 12 h dark cycle. All the animals were provided laboratory rodent diet (Nutrilab Rodent diet, Vetcare, Netherlands) except for 2 h before treatment and 2 h after the drug administration. Reverse osmosis water treated with ultraviolet light was provided ad libitum.

Methods

Formulation Preparation
Rosuvastatin was prepared in a vehicle of normal saline for oral and i.v. administration in a final concentration of 1 mg of rosuvastatin/ml for i.v and 2.5 mg of rosuvastatin/ml for oral. For piperine, 20% ethanol /40% propylene glycol/40% Milli q water (v/v/v) were used as a vehicle for oral administration in a final concentration of 1 mg of piperine/ml. Cinnamic acid was prepared in a vehicle of 10 % ethanol/90 % Milli Q Water (v/v) for oral administration in a final concentration of 1 mg of cinnamic acid/ml. Gallic acid for oral administration was prepared by dissolving gallic acid in a vehicle of 10% ethanol/90% Milli Q Water (v/v) for oral. For piperine, 20% ethanol /40% propylene glycol (lot no. #1359720) and formic acid (lot no. # BCBC4518V) were procured from Sigma-Aldrich (Aldrich, St. Louis, MO). Acetonitrile and methanol were of HPLC grade procured from JT Baker (Phillipsburg, NJ, USA). Demineralized water was prepared in-house using milli-pore water system (USA). Absolute ethanol (99.9%) was purchased from Tedia Company, Inc (Fairfield, OH, USA). All the other reagents or solvents used were either analytical or high-performance liquid chromatography (HPLC) grade.

Dose Administration
Rosuvastatin was freshly prepared in Milli Q for intravenous (i.v.) and per oral (p.o.) dosage. Eight groups of mice were formed and each group having nine mice (n=9). Four groups of nine animals each were given 5 mg/kg rosvastatin intravenously, alone or co-administered (30 minutes prior) with piperine or gallic acid or cinnamic acid (5 mg/kg) by per oral (p.o.) administration through 16-gauge stainless steel needle. The other four groups of nine animals each were given 25 mg/kg rosuvastatin orally, alone or co-administered (30 minutes prior) with piperine or gallic acid or cinnamic acid (5 mg/kg) by oral gavage using 16-gauge stainless steel needle. Rosuvastatin doses (5 and 25 mg/kg) were chosen to keep plasma concentrations above the limit of detection at the time variation from 0 to 48 h in mice plasma. The dose (5 and 25 mg/kg) of co-administered drugs (piperine, gallic acid and cinnamic) was chosen at 5 mg/kg since the maximal bioenhancement effect was observed at this dose (in-house study, data not shown). Mice were fasted overnight and the fasting continued up to 2 h post dosing with free access to drinking water.

For i.v. drug interaction studies, a dose of 5 mg/kg piperine or gallic acid or cinnamic acid was first given separately to the respective group of mice via oral gavage using 16-Gauge stainless steel needle (p.o.). Thirty minutes later, a dose of 5 mg/kg rosuvastatin was given to the mice via tail vein injection. Nine mice were used for each group.

For per oral (p.o.) drug interaction studies, a specified dose (5 mg/kg) of the test compounds (piperine, gallic acid and cinnamic acid, solution formation) was administered separately to the respective group of mice by oral gavage using 16-gauge stainless steel needle. Thirty minutes later, the animals were administered 25 mg/kg rosuvastatin through per oral (p.o.) route. Nine mice were used for each group.

Sample Collection and Processing
Blood samples (~60 µL) were collected using sparse from the retro-orbital plexus into labeled tubes, containing 10 µL of K$_2$EDTA solution (20%), as anticoagulant. Blood samples from intravenous (i.v.) animals were collected at 0.08, 0.25, 0.5, 1, 2, 4, 8, 24 and 48 h post-dose and from per oral (p.o.) animals, at 0.5, 1, 2, 4, 8, 24 and 48 h post-dose. Pre-dose samples were collected from a different group of untreated animals. The blood samples were collected using sparse sampling design. Plasma was harvested from the blood by centrifugation at 4000 rpm for 10 min at 4 ± 2 °C and stored below -70 °C (Thermo Scientific, USA) deep freezer until bioanalysis.

Analytical Methodology
Plasma (25 µl) protein was precipitated by using 100 µl acetonitrile containing internal standard (Atorvastatin calcium 200 ng/ml in acetonitrile). Vortexed the sample for 5 minutes, after centrifugation at 15000 rpm for 10 min, 100 µl of supernatant was transferred to clean insert vial and analyzed by liquid chromatography (LC) with tandem mass spectrometry detection. A Shimadzu LC-20AD LC system (Kyoto, Japan) was connected to a SIL HTC auto-sampler (Kyoto, Japan). The supernatant were injected (5 µl) onto a 75 x 4.6 mm (3.5 µm) Waters SymmetryShield RP 18 column (Waters, Massachusetts, Ireland). Analystes were eluted using a gradient elution program with a mobile phase consists of acetonitrile (pump A) with 5 mM ammonium acetate with 0.1% formic acid (pump B) at a flow rate of 0.8 ml/min. The column temperature was 40 °C, the sample temperature was 4 °C. The following linear gradient was employed for the separation: 30% A for 1 min, 90% A at 2 min, and hold to 2.5 min, 30% A at 3.5 min and hold to 4.5 min. The rosvastatin and atorvastatin elution times were ~1.97 and 2.21 min, respectively.

Assay was performed on API 4000 Applied Biosystem Sciex LC/MS/MS (Concord, Ontario, Canada) triple quadrupole mass analyzer system with an turbo ion spray atmospheric pressure ionization interface connected to a Shimadzu LC-20AD LC system (Shimadzu Corp., Japan). The instrument was operated in the multiple reaction monitoring mode (MRM). The best sensitivities and minimum interferences were achieved by monitoring the molecular precursor and daughter ions, for rosuvastatin m/z 482.3 → 258.3, 482.3 → 300.1, 482.3 → 270.4, 482.3 → 188.9 and similarly for atorvastatin (IS) m/z 559.3 → 466.3, 559.3 → 440.5, 559.3 → 292.4 and 559.3 → 250.3. The optimum operating parameters of electro spray ionization (ESI) interface in positive ion mode were: interface temperature 550 °C, nebulizing gas 60 psi, drying gas 55 psi and detector ESI probe voltage 5000 volts. The voltage parameters like DP, CE, CXP, dwell time were optimized for each analyte and fragment to get better sensitivity.

A mouse plasma calibration curve range starting from ~1 - 5000 ng/ml was selected. Calibration curves were derived from peak area ratios (analyte/ internal standard) using a least-squares regression of the ratio versus the nominal concentration of the calibration curve standard. The whole system
was controlled by Analyst Classic 1.5 software (Applied-Biosystem-Sciex, Concord Canada). Stock solutions of rosuvastatin, and atorvastatin (internal standard, IS) were prepared in methanol at approximately 1 mg/ml and subsequently diluted and used for spiked blank matrices for construction of calibration samples and quality control samples.

**Pharmacokinetic Analysis**

The pharmacokinetic parameters of rosuvastatin were obtained by non-compartmental analysis using WinNonlin version 2.1 (Pharsight, Mountain View, CA). The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal method. The terminal half-life ($t_{1/2}$) was calculated as $\ln(2)/k$, and $k$ was determined from the slope of the terminal regression line. The systemic clearance (CL) and apparent oral clearance (CL/F) were calculated as the i.v. and p.o. dose divided by AUC, respectively. Non-compartmental analysis using WinNonlin version 2.1 (Pharsight, Mountain View, CA) was used to assess the pharmacokinetic parameters. Area under the cumulative curve was evaluated by non-compartmental analysis. Non-Compartmental-Analysis (NCA) was taken into consideration when clearance was determined for the oral route (CL/F). The absolute bioavailability (F) was determined by $(\text{AUC}_{\text{p.o.}}/\text{Dose}_{\text{p.o.}})/(\text{AUC}_{\text{i.v.}}/\text{Dose}_{\text{i.v.}})$.

**RESULTS AND DISCUSSION**

Pharmacokinetics of rosuvastatin was evaluated in healthy male C57 BL/6 mice following single 5 mg/kg intravenous or 25 mg/kg oral dose administration either alone or combination with bioenhancer (P.O, 5 mg/kg) viz. piperine, and herbal compounds viz. gallic acid and cinnamic acid. Intravenous dose was administered via tail vein at a steady rate over 50-60 second. The oral dose was given as a gavage. The formulations of rosuvastatin were prepared as solution in normal saline. Oral herbal formulations were prepared as solution such as piperine solution using 20% ethanol, 40% PG, 40% milli q water, gallic acid using milli q water and cinnamic acid using 10% ethanol and 90 % milli q water. Oral herbal solution (5 mg/kg) was administered to respective group 30 minutes before rosuvastatin intravenous and oral administration. The detail of the treatment groups is given in Table 1. The blood samples were collected using sparse sampling design up to 48 h post-dose from retro-orbital plexus. Pharmacokinetic parameters were evaluated by non-compartmental analysis. Non-Compartmental-Analysis module in WinNonlin® version 5.2 (Pharsight Corporation, Mountain View, CA) was used to assess the pharmacokinetic parameters. Area under the plasma concentration versus time curve (AUC) up to 48 h was determined by the linear trapezoidal rule.

**Table 1: Treatment groups.**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Sub-division</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>Rosuvastatin</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>Alone</td>
<td>(Intravenous)</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>Rosuvastatin</td>
<td>(Intravenous)</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>Co-administration</td>
<td>(Intravenous)</td>
<td>&amp;</td>
</tr>
<tr>
<td>1</td>
<td>with Piperine</td>
<td>&amp;Piperine</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Rosuvastatin</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Rosuvastatin</td>
<td>(Intravenous)</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Co-administration</td>
<td>(Intravenous)</td>
<td>&amp;</td>
</tr>
<tr>
<td>2</td>
<td>with Gallic Acid</td>
<td>&amp;Gallic Acid</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Cinnamic Acid</td>
<td>&amp; Cinnamic Acid</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 3: Pharmacokinetic parameters of rosuvastatin (RSV) in male C57BL/6 mice following single (5 mg/kg) intravenous (LV) dose and co-administration with per oral dose (5 mg/kg) of PIP, GA and CA**

<table>
<thead>
<tr>
<th>Route</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Formulation</th>
<th>$C_{\text{ss}}$ (mg/mL)</th>
<th>$T_{\text{ss}}$ (hr)</th>
<th>$AUC_{\text{ss}}$ (mg/h)</th>
<th>$V_{\text{ss}}$ (L/kg)</th>
<th>Last $T_{\text{ss}}$ (hr)</th>
<th>$MRT_{\text{ss}}$ (hr)</th>
<th>Clearance (mL/min/kg)</th>
<th>$V_{\text{ss}}$ (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
<td>RSV Alone</td>
<td>5</td>
<td>Solution</td>
<td>2307.68</td>
<td>5.38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LV</td>
<td>RSV + PIP</td>
<td>5</td>
<td>Solution 5</td>
<td>6226.94</td>
<td>4.26</td>
<td>1108.47</td>
<td>70.91</td>
<td>4.18</td>
<td>4.76</td>
<td>70.91</td>
<td>38.58</td>
</tr>
<tr>
<td>LV</td>
<td>RSV + GA</td>
<td>5</td>
<td>Solution 5</td>
<td>6078.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LV</td>
<td>RSV + CA</td>
<td>5</td>
<td>Solution 5</td>
<td>6078.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^{c}$ Back extrapolated concentration of IV profile.

**Effects of piperine, gallic acid and cinnamic acid on rosuvastatin pharmacokinetics in C57 BL/6 mice after per oral administration**

The plasma concentration–time profiles of rosuvastatin after oral (25 mg/kg) administration in the absence or presence of oral co-administered of piperine, gallic acid and cinnamic acid (5 mg/kg) were illustrated in Figure 2.
and the pharmacokinetic parameters for rosuvastatin were shown in Table 4. The presence of 5 mg/kg piperine, gallic acid and cinnamic acid significantly increased the AUC\(_{\text{last}}\) of rosuvastatin increased by 34.90%, 66.03% and 62.49%, respectively. Consequently, the relative exposure (AUC\(_{\text{last}}\)) of rosuvastatin was increased by 1.35, 1.66 and 1.62-fold in the presence of 5 mg/kg piperine, gallic acid and cinnamic acid, respectively. The oral co-administered of piperine, cinnamic acid and gallic acid (5 mg/kg) also increased significantly the peak plasma concentration (C\(_{\text{max}}\)) of rosuvastatin by 10.39 %, 52.63 % and 42.18 %, respectively. The apparent oral plasma clearance (CL/F) of rosuvastatin was decreased significantly by 35.14%, 35.59%, 34.09 % in the presence of 5 mg/kg piperine, gallic acid and cinnamic acid, respectively. Oral t\(_{1/2}\) of rosuvastatin along was 7.3 h and in the presence of piperine, gallic acid and cinnamic acid 8.0, 6.9 and 3.8 h respectively was observed.

Figure 2: Mean plasma concentration profile of rosuvastatin (RSV) in male C57BL/6 mice following single (25 mg/kg) per oral (P.O.) dose and co-administration with per oral dose (5 mg/kg) of PIP, GA and CA.

Table 4: Pharmacokinetic parameters of rosuvastatin (RSV) in male C57BL/6 mice following single (25 mg/kg) per oral (P.O.) dose and co-administration with per oral dose (5 mg/kg) of PIP, GA and CA.

<table>
<thead>
<tr>
<th>Route</th>
<th>P.O.</th>
<th>P.O.</th>
<th>P.O.</th>
<th>P.O.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>RSV Alone</td>
<td>RSV + PIP</td>
<td>RSV + GA</td>
<td>RSV + CA</td>
</tr>
<tr>
<td>RSV Dose (mg/kg)</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Formulation</td>
<td>Solution</td>
<td>Solution</td>
<td>Solution</td>
<td>Solution</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/mL)</td>
<td>799.09</td>
<td>716.26</td>
<td>1019.15</td>
<td>1136.41</td>
</tr>
<tr>
<td>T(_{1/2}) (h)</td>
<td>0.25</td>
<td>0.50</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>aAUC(_{\text{last}}) (ng/mL*h)</td>
<td>735.54</td>
<td>992.23</td>
<td>1221.25</td>
<td>1195.16</td>
</tr>
<tr>
<td>aAUC(_{\text{0-24h}}) (ng/mL*h)</td>
<td>741.46</td>
<td>1006.13</td>
<td>1251.32</td>
<td>1200.01</td>
</tr>
<tr>
<td>Clearance (CL/P) (mL/min/kg)</td>
<td>3.73</td>
<td>8.0</td>
<td>6.9</td>
<td>3.8</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>33</td>
<td>45</td>
<td>56</td>
<td>54</td>
</tr>
</tbody>
</table>

\(a\) AUC\(_{\text{last}}\) considered for bioavailability calculation. RSV indicates rosuvastatin, PIP is Piperine, GA is Gallic acid and CA indicates Cinnamic acid.

The observed absolute oral bioavailability of rosuvastatin alone is 33% whereas rosuvastatin co-administered with per oral dose of piperine, gallic acid and cinnamic acid is 45, 56 and 54%, respectively.

**LC–MS/MS optimization**

Rosuvastatin in plasma was measured by LC-MS/MS method. APi 4000 Applied Biosystem-Sciex LC/MS/MS (Concord, Ontario, Canada) triple quadruple mass analyzer system with an turbo ion spray atmospheric pressure ionization interface coupled with a Shimadzu LC-20AD LC system (Shimadzu Corp., Japan). When rosuvastatin tuned with flow injection analysis (FIA) using single standard solution, obvious protonated molecules [M + H]\(^+\) were observed in Q1 full-scan. Then fragments of protonated molecules were obtained in product ion scan at collision cell. Multiple reactions monitoring mode (MRM) scan was used for quantitation of all analytes. In order to obtain the intensity in signal, sum of prominent products ion were selected. Compound dependant parameters including declustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) were optimized to achieve highest intensity for each intense fragment. Detailed parameters were summarized in Table 2. The optimum operating parameters of EelectroSpray ionization (ESI) interface in positive ion mode were: Interface temperature 550 °C, Nebulizing gas 60 psi, Drying gas 55 psi and detector ESI probe voltage 5000 volts. Care was taken to adjust dwell times and delays between ion transitions so that there are at least ten to twelve data points for each monitored peak. Failure to meet this requirement may result in inadequate peak integration and considerably worse precision.\(^[31]\) A dwell time of 50 ms for each MS/MS transition was used. It was difficult to find a compound, which could ideally mirror all three analytes to serve as a good IS. Several compounds were investigated to find a suitable IS, and finally atorvastatin was found to be the most appropriate for the present study. There was no significant effect of IS on analytes recovery, sensitivity or ion suppression was observed. Chromatographic separation was carried out using 75 x 4.6 mm (3.5 µm) Waters SymmetryShield RP 18 column. Peak integration, regeneration and calculation of concentration were computed using Analyst Classic (Version 1.5) software. A weighting of 1/x\(^2\) where x is the concentration of a given calibration standard level was found to be optimal. Representative LC-MS/MS chromatogram of rosuvastatin using atorvastatin as an internal standard (IS) in male C57BL/6 mice plasma shown in Figure 3.

Table 2: Positive product ion mass parameter of rosuvastatin and atorvastatin (IS).

<table>
<thead>
<tr>
<th>Name of the Analyte</th>
<th>Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>DP (msec)</th>
<th>Dwell time (msec)</th>
<th>CE</th>
<th>CXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>482.3</td>
<td>258.3</td>
<td>123.0</td>
<td>100</td>
<td>47.08</td>
<td>5.67</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>559.3</td>
<td>466.3</td>
<td>101.0</td>
<td>100</td>
<td>24.22</td>
<td>6.04</td>
</tr>
<tr>
<td></td>
<td>559.3</td>
<td>440.5</td>
<td>30.32</td>
<td>5.99</td>
<td>46.00</td>
<td>6.18</td>
</tr>
<tr>
<td></td>
<td>559.3</td>
<td>292.4</td>
<td>63.20</td>
<td>6.20</td>
<td>EP: 10</td>
<td></td>
</tr>
</tbody>
</table>

DP indicates Declustering Potential, CE is collision energy, CXP is Cell Exist Potential and EP indicates Entrance Potential. All are voltage dependent parameters of instrument.
CONCLUSION

Co-administered of herbal with medicinal drugs is frequent and the likelihood of a clinical relevant interaction is high[21]. Despite of the data shown by most of the literature it is likely that such interactions are common than generally thought, but are under-reported and appearance of adverse effects may be attributed to the disease for which the treatment is taken. Therefore, interaction between herbal drugs and medicinal drugs are not any more just a theoretical possibility. There are many sources of phytochemicals, including herbal medicines, vegetables and food materials and it is not feasible to check the intake. Therefore knowingly or unknowingly these agents act on the physiological targets resulting in herb-drug interaction and any untoward effect is considered as adverse effect of drug. Piperine has been shown in clinical trials to increase plasma concentration of phenytoin, propanolol, and theophylline[19, 20]. Poly phenols (cinnamic acid and gallic acid) present in many fruits and vegetables[19]. These herbal medicines or bioenhancers (piperine, gallic acid and cinnamic acid) were not investigated for its ability to increase bioavailability of rosuvastatin.

Co-administered of drug candidates with herbal medicines, which might be the inhibitors of known drug transporters and metabolizing enzymes, may represent a strategy to improve the bioavailability of the co-administered drug. The mechanisms underlying most of the reported herb-drug interactions have been ascribed to the inhibition of various types of efflux transporters such as P-glycoprotein (P-gp), multi drug resistance proteins (MRPs), organic anionic transporter polypeptides (OATPs), taurocholate cotransporting polypeptide (NTCP), breast cancer resistance protein (BCRP) and/or drug-metabolizing enzymes, cytochrome P450s (CYPs)[14–19]. The piperine, cinnamic acid and gallic acid were selected for this study, since little or no information is available in the literature of their used either as CYP inhibitor and/or efflux transporters.

The objective of this study was to examine the potential pharmacokinetic interaction between piperine and rosuvastatin, or cinnamic acid and rosuvastatin or gallic acid and rosuvastatin in mice. In this report, we describe, for the first time in mice, the effects of piperine, gallic acid and cinnamic acid on the pharmacokinetics of rosuvastatin. After co-administered with per oral dose of (5mg/kg) piperine, gallic acid and cinnamic acid the relative exposure (AUC last) of rosuvastatin increased by 2.75, 2.77 and 2.81 fold in case of intravenous administration (5mg/kg). There is 1.3, 1.7, 1.6 fold increase in AUC after per oral (25 mg/kg) administration rosuvastatin, when co-administered with per oral dose of 5mg/kg piperine, gallic acid and cinnamic acid, respectively. The absolute oral bioavailability of rosuvastatin in mice observed alone is 33 % whereas rosuvastatin co-administered with piperine, gallic acid and cinnamic acid is 45, 56 and 54%, respectively. Hence there was 15, 19 and 18 % increase in oral availability of rosuvastatin when co-administered with piperine, gallic acid and cinnamic acid in C57BL/6 male mice. Piperine, gallic acid and cinnamic acid can acts as herbal bioenhancer but need to evaluate for some other drugs too. There mechanism of action for enhancing the bioavailability is not clearly know and can be evaluated.

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