Repercussion of piperine on pharmacokinetics parameters of eletriptan in albino rats: Involvement of CYP3A and P-GP inhibition

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ABSTRACT

Eletriptan is at the type of triptan medication suggested for the management of episodes of severe headache with or without aura. Piperine (1-piperoylpiperidine) is a pure alkaloid as well as the basic tanginess material from the pipli and peppercorn. There are signs that piperine inhibits Cytochrome P-450 enzymes and P-glycoprotein, rather than arouses, drug metabolism generally, thus increasing the bioavailability and effect of several medications. The analysis was undertaken to assess the results of Piperine on the pharmacokinetics of Eletriptan. Samples of blood were taken at different time points for example 0 (predose), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 12, 24 hours post-treatment. The plasma concentration of Eletriptan was estimated with the HPLC procedure. In our study Cmax, Tmax, AUC0-24 , AUC0-8, AUC%, AUMC0-24, AUMC0-8, t1/2, MRT0-24, MRT0-8 and Volume of distribution were increased by approximately 51.76%, 7.55%, 72.93%, 84.42%, 42.72%, 93.56%, 128.82%, 19.97%, 11.74%, 23.96% and 3% respectively, whereas clearance decreased by 42.10% when eletriptan co-administered with piperine. In summary, the results obtained herein imply that Piperine is improving the bioavailability of Eletriptan by strengthening the exposure (AUC) of their Eletriptan when concomitantly administered by the oral route. The Piperine improved the oral bioavailability of eletriptan by inhibiting CYP3A and P-GP in rats. This observation indicates the possibility that the combo of piperine along with other CYP3A and P-GP double substrates can also enhance bioavailability.

INTRODUCTION

Oral absorption of medications is a really important issue particularly when the medication is poorly bioavailable, provided for extended periods, and pricey. Indisposed bioavailability of drugs stay subtherapeutic as a big a part of a dose not enter into the blood plasma or strive its biological effect unless and until quite huge doses are as long as can also end in severe side effects. Every substantial upgrading in bioavailability goes to steer to lowering the dose or the dosage occurrence of that specific molecule. Several approaches are utilized to
optimize oral bioavailability, but with the discovery of this very first bioavailability enhancer piperine in 1979, a new category of drugs along with a new theory was introduced to the science.

Bioenhancers or bioavailability enhancers are largely the plant-based molecules which encourage the biological action or bioavailability or the uptake of drugs in combination treatment (Wadhwa et al., 2014). Piperine (PIP) is a nutritional supplement alkaloid, a significant part of black pepper (Piper nigrum Linn), also continues to be worn as a nutrient and spice booster. Piperine performed quite a few health-benefiting effects, like free radical scavenging, anti-mutagenic, healing of ulcers, anti-inflammatorv and antitumor actions (Srinivasan, 2007; Meghwal and Goswami, 2013).

Precedent research have revealed that piperine improves the bioavailability of several bodily and pharmacological assorted drugs, including phenytoin (Bano et al., 1987; Velpandian et al., 2001; Pattanaik et al., 2006)-RBB-, carbamazepine (Pattanaik et al., 2009), midazolam (Rezaee et al., 2014), propranolol and theophylline (Bano et al., 1991). Piperine was reveal to work as an blockade of allele mediated by P-glycoprotein (P-gp), an efflux membrane transporter, and numerous cytochrome P450 enzymes (CYP) on top of phase II metabolism (Han et al., 2008; Koul et al., 2000).

In vitro results of the studies moreover designate that piperine inhibits CYP3A4, CYP2C9 and UDP glucuronyl transferase dependent metabolism (Volak et al., 2014). It was also revealed to amend the pharmacokinetics of the P-gp and domperidone substrate of CYP (Alhumayyd et al., 2014) and fexofenadine (Jin and Han, 2010) in rats via reticence of P-gp-mediated drug transport. Eletriptan is a second-generation triptan medication meant for treating migraine headaches.

The eletriptan bioavailability was reported to be around 40-50%. The eletriptan native sensible oral bioavailability was due to drug degrading enzymes (CYP3A) and outflow carriers (P-gp) which are located in epithelial cells (enterocytes) lining of small intestine and in the parenchymal cell of the liver (hepatocytes) (Heykants et al., 1981).

Consequently, orally administered Eletriptan can be metabolized twofold prior to getting the systemic circulation. Thus, oral bioavailability can be obviously attenuated. Present investigation, we deliberate that if Piperine acts as a blockade of CYP3A and P-gp via drug outflow, possibly in the liver and intestine, it will augment the bioavailability of Eletriptan, a CYP3A and P-gp substrate (Neerati and Yakkanti, 2014). To our understanding, there are no available information regarding to interaction between Piperine and eletriptan. Hence, we tested the feasible impact of Piperine management on pharmacokinetic of CYP3A and P-gp arbitrates drug outflux using eletriptan as a CYP3A, P-gp substrate in Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals

Eletriptan Hydrobromide was acquired from Pfizer Laboratories Hyderabad (India); as a gift sample. Piperine was bought from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. All of HPLC grade solvents (acetonitrile, Sodium Lauryl Sulfate, Ortho Phosphoric Acid and water) were grab from R.K Fine laboratories, Gunter, India. The additional chemicals were acquired from neighboring compound bureaus.

Animals

The animals (Albino Wistar rats) weighing between 200 to 250 gram of either sex, were chosen and acquired from NIN, Hyderabad, India. Animals were reserved as per regular laboratory environment at 24.5 ± 2.5°C, relative humidity 50-40 ± 15%, and standard day period (12 h gloomy/12 h daylight). Rat pellet diet and water were supplied ad lib. The investigational procedure was official by the Institutional Animal Ethics Committee at Nova College of Pharmacy bearing registration number 1747/PO/Re/S/14/CPCSEA and research have been completed according to the principles of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), India.

Pharmacokinetic study in rats

Preparation of drugs

Eletriptan hydrobromide was liquefy in distilled water at which as piperine (10mg) was correctly weighed prior to being triturated at a sterile wash mortar with the accession of 30μL of tween 80 and subsequently, demanded quantity of 0.9% sodium CMC was inserted and triturated again to freeze the medication within it. Subsequently, the suspension was transferred to plastic vials. After preparation of Piperine suspension within 10 minutes was dispensed concurrently with Eletriptan Hydrobromide solution to the animals.

Investigational process

The selected animals were arbitrarily scattered into two groups and each group have six animals. Prior to dosing, all grouped animals have abstained for 16 h and however, the water was given ad lib. The study protocol design was as follows.
Group I: Treated with 2mg/kg body weight of eletriptan solution orally.

Group II: Treated concurrently with Eletriptan Hydrobromide solution (2 mg/kg body weight) and Piperine suspension (10 mg/kg body weight) orally.

**Blood sample collection in rats**

Within this analysis, blood specimen were collected into sodium citrate containing eppendorf (2ml) from retroorbital sinuses at time points 0 (predose), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 12, 24 hours post treatment. Plasma was alienated by centrifugation at 6000 RPM/15 minutes, kept at -25°C further additional investigation. The plasma concentration of Eletriptan was projected with a sensitive HPLC technique.

**Assessment of Eletriptan in plasma by a sensitive HPLC method**

The HPLC technique developed was also employed for its estimation of eletriptan in plasma samples with the internal standard procedure. For this function, a calibration curve was constructed by assessing plasma samples containing various concentrations of eletriptan.

**Standard solutions**

The main stock solution of 1mg/ml of eletriptan was prepared in methanol. Appropriate dilutions of eletriptan from stock options were produced at the mobile phase to create working stock options of 0.05, 0.10, 0.25, 0.50, 1, 2, and 4 μg/ml. Those dilutions were used to spike plasma at the preparation of calibration of this curve. Eletriptan spiked plasma samples were prepared by mixing 1 tsp clean plasma with proper volumes of the conventional eletriptan solutions (100μL) on the afternoon of this analysis. A blank was prepared to comprise 1 mL plasma.

**Extraction procedure**

Plasma was lanced with varying amounts of eletriptan stock solution was ready, in order to provide a run drug concentrations ranging from 0.05 to 4 μg/kg. 125μL of compacted plasma was obtained and also to the 25μL of internal standard (Topiramate inventory alternative 10μg/ml in methanol) was added and then vortexed (Vortex mixer, Genei, Mumbai) for 60 minutes. Subsequently 500μL of methanol was added to precipitate proteins and vortexed for 5 min and centrifuged at 5000 rpm in a microcentrifuge (REMI Scientiﬁcs, India) for 10 min. The supernatant was removed and taken in a vacuum oven at 40°C. Dried samples were then redispersed in 100μL methanol and vortexed. The upper layer was shifted into a tubes of microcentrifuge and out of this 20μL have recovered for HPLC analysis.

**Chromatographic conditions**

Quaternary gradient HPLC (Waters Delta prep HPLC system, USA) using a rheodyne guide injector (Rheodyne, Cotati, CA, USA) attached using a 100μL sample loop has been used for loading the sample. A varying wavelength programmable photodiode array (PDA) detector (Waters 2999 PDA, USA) and reversed-phase C-18 pillar undefined was utilized. The HPLC system that was armed with the EMPOWER two applications (Waters, Milford, MA, USA) was utilized for data processing and acquisition. Acetonitrile and phosphate buffer (15mM) used as a mobile phase. The mobile phase pH was adjusted to 3.5±0.1 with orthophosphoric acid. The filtered mobile phase components were pumped out of the various reservoirs in the pressure rate of 1ml/min. The temperature of column was kept at normal temperature (30°C). The eluent was discovered by a PDA detector at a wavelength of 236 nm.

**Pharmacokinetic data analysis**

Pharmacokinetic parameters were calculated with the Try-Kinetica applications trial version 5.0. When”NCA assistant-non-compartmental-extra vascular” webpage was started. Components of time concentrations and points have been awarded. After the information was entered, assess button has been clicked and then a chart was looked. Study button has been pressed to see couple of PK parameters. Afterward, data was analyzed by clicking analyze. Then, two-line charts were seemed. Then, study choice was clicked to find the comprehensive PK parameters of Eletriptan hydrobromide. Each creature data was granted and PK parameters were computed for each animal information. Afterward, average of a single PK parameter for the animals of an exact same group has been shot. This information was subjected to statistical evaluation.

**Statistical Analysis**

The values of present study were conveyed as mean ± S.D. The Eletriptan Hydrobromide-alone group plasma concentration vs. time profile was compared with Eletriptan Hydrobromide using the piperine mix group were examined using 2 way ANOVA succeed by Bonferroni post hoc evaluation whereas contrasts of the pharmacokinetic parameters of both of these classes were inspected by unpaired student’s t test. *P<0.05, **P<0.01, ***P<0.001 were regarded to be statistically significant.

**RESULTS**

**Calibration curve**

The run time was put in 10min and eletriptan and
inner standard appeared about the chromatogram in 3.109 minutes and 8.502 respectively as shown in Figures 1, 2 and 3. There was no hindrance of any other summit with a medication summit. When the exact same sample comprising the medication was injected six times, the retention period of this medication was nearly the exact same for the six injection samples. The mean peak region of eletriptan and its summit areas were subjected to regression analysis by the least square method, and also a higher correlation coefficient was detected ($r=0.994$) at the array of 0.05-4 µg/mL. This regression equation was utilized to gauge the sum of eletriptan in plasma. Calibration values have been revealed in Table 1 and the Linearity chart was revealed at Figure 4.

Figure 1: HPLC chromatogram of blank plasma

Figure 2: HPLC chromatogram of blank plasma with internal standard (Topiramate)

Figure 3: HPLC chromatogram of spiked plasma with Eletriptan

Figure 4: Calibration curve for the estimation of eletriptan in plasma

Effect of Piperine on plasma concentration time profiles of Eletriptan

The Table 2 and Figure 5 showed time profiles vs plasma concentrations of oral treatment of Eletriptan Hydrobromide with and without piperine in rats. From the comparison of eletriptan in the absence and presence of piperine plasma concentration profiles, it is clear that there was a significant increase in the plasma drug exposure of Eletriptan in the combination group at following time points 0.5hr ($^{ns} P > 0.05$), 1.0 hr ($^{ns} P > 0.05$), 1.5 hr ($^{**} P > 0.05$), 2.0 hr ($^{***} P < 0.001$), 2.5 hr ($^{***} P < 0.001$), 3.0 hr ($^{***} P < 0.001$), 3.5 hr ($^{***} P < 0.001$), 4th hr ($^{**} P > 0.001$), 6.0 hr ($^{***} P < 0.001$), 8.0 hr ($^{***} P > 0.001$), 12.0 hr ($^{*} P < 0.05$) and 24.0 hr ($^{ns} P > 0.05$).

Figure 5: Comparison of mean plasma concentrations of Eletriptan treated group and eletriptan with Piperine treated group – single dose study

Effect of Piperine on pharmacokinetic parameters of Eletriptan

The calculated pharmacokinetic parameters of Eletriptan Hydrobromide and Eletriptan Hydrobromide with piperine in rats were shown in Table 3. The $AUC_{0-24}$ of Eletriptan has significantly ($P<0.001$) increased in the combination group (1567.18 ± 67.25$^{***}$) than $AUC_{0-24}$ of Eletriptan of Eletriptan alone treated group (906.20±34.44). This increase is almost 1.7 times. In similar manner, the $C_{max}$ of Eletriptan has significantly ($P<0.01$) increased in the combination group (207.27±5.5$^{***}$) than $C_{max}$ of Eletriptan of Eletriptan alone treated group (136.57±3.50). This increase is almost 1.5 times. Compared with the eletriptan alone group, the $AUMC$, $MRT$, $t_{1/2}$ significantly ($P<0.001$) increased when eletriptan combined with piperine (combination group). In contrast, the clearance of combined group (0.0011±0.0005$^{***}$) significantly ($P<0.001$) decreased when compared with eletriptan alone group (0.0019±0.0001). The $t_{max}$ and volume of distribution of combined group not significantly altered when compared with alone group. The pharmacokinetic parameters were represented in Table 3 and expressed in Figure 6.
### Table 1: Calibration of the HPLC method for the estimation of eletriptan in plasma by using topiramate as internal standard

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Plasma concentration of Eletriptan (µg/ml)</th>
<th>Mean peak area of Eletriptan</th>
<th>Mean peak area of internal standard</th>
<th>Mean peak area ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>69701.45</td>
<td>435212.81</td>
<td>0.160</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>96378.64</td>
<td>428349.52</td>
<td>0.225</td>
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<tr>
<td>3</td>
<td>0.25</td>
<td>202790.34</td>
<td>429855.78</td>
<td>0.472</td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
<td>367206.29</td>
<td>425007.28</td>
<td>0.864</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>584666.07</td>
<td>433728.54</td>
<td>1.348</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1149978.73</td>
<td>428776.56</td>
<td>2.682</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>1953184.27</td>
<td>421289.19</td>
<td>4.636</td>
</tr>
</tbody>
</table>

### Table 2: Comparison of mean concentrations of eletriptan treated group and eletriptan with piperine treated group – single dose study

<table>
<thead>
<tr>
<th>Time Points (hr)</th>
<th>Eletriptan (2mg/kg)</th>
<th>Eletriptan (2mg/kg) + Piperine (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.E.M</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>48.81</td>
<td>6.10</td>
</tr>
<tr>
<td>1</td>
<td>81.13</td>
<td>5.68</td>
</tr>
<tr>
<td>1.5</td>
<td>109.38</td>
<td>6.70</td>
</tr>
<tr>
<td>2</td>
<td>124.91</td>
<td>4.66</td>
</tr>
<tr>
<td>2.5</td>
<td>127.57</td>
<td>7.53</td>
</tr>
<tr>
<td>3</td>
<td>103.92</td>
<td>6.97</td>
</tr>
<tr>
<td>3.5</td>
<td>88.65</td>
<td>6.90</td>
</tr>
<tr>
<td>4</td>
<td>72.08</td>
<td>5.40</td>
</tr>
<tr>
<td>6</td>
<td>52.53</td>
<td>3.69</td>
</tr>
<tr>
<td>8</td>
<td>38.34</td>
<td>1.69</td>
</tr>
<tr>
<td>12</td>
<td>24.88</td>
<td>1.87</td>
</tr>
<tr>
<td>24</td>
<td>11.85</td>
<td>1.10</td>
</tr>
</tbody>
</table>

![Figure 6: Comparison of pharmacokinetic parameters of Eletriptan alone group and Eletriptan with Piperine combination group – single dose study](image-url)
The percentage change of various pharmacokinetic parameters like Cmax, Tmax, AUC0–24, AUC0–∞, AUC%, AUMC0–24, AUMC0–∞, t1/2, MRT0–24, MRT0–∞ and Volume of distribution were increased by approximately 51.76%, 7.55%, 72.93%, 84.42%, 42.72%, 93.56%, 128.82%, 19.97%, 11.74%, 23.96% and 3% respectively, where as clearance decreased by 42.10% after treatment with piperine.

### DISCUSSION

Foods and dietary add-on such as spices, herbs, teas, fruits, and veggies have multipart mixtures of plant chemicals which have a best capacity to excite or control the expression and action of drug-metabolizing enzymes. CYP450 enzymes might be especially susceptible to inflection from the multiple dynamic components of foods, such as nutritional complements (Bailey et al., 2003). CYP3A series enzymes have been known to participate in the most typical food-drug interactions, according to reports of clinically significant interactions between orally administered drugs which are substrates of the enzyme (Harris et al., 2003).

The greater bioavailability is because of decreased metabolism which in turn might be due to a combined result of inhibition of metabolizing enzymes belonging to the CYP group and inhibition of the efflux transporter P-GP. Such improved absorption was reported previously by most researchers for various medications with pretreatment with plant compounds including anthocyanins, polyphenols, and flavonoids (Won et al., 2010).

By the past ten years, using phytochemicals together with medication as complementary and alternative medicine is growing tremendously considering they are safe with minimal negative consequences. These phytochemicals can modulate different drug transporter systems and drug-metabolizing enzymes that result in potential herbal-drug interactions (Hermann and Richter, 2012; Zhang et al., 2003). Piperine, the most important alkaloid present in black pepper and honey reveal blocked effects on drug out flux transporter (P-GP) and among the significant drug-metabolizing enzymes CYP3A. Piperine was reported to demonstrate an inhibitory influence on P-GP mediated efflux of Digoxin and cyclosporine in Caco-2 cells and CYP3A4 mediated breakdown of Verapamil in liver microsomes of human (Bhardwaj et al., 2002).

The present investigation, the effect of piperine pretreatment on the oral bioavailability of eletriptan has been detected. The interaction between eletriptan and piperine was analyzed with an in-vivo rat version. The outcomes in the in-vivo oral pharmacokinetic study demonstrated a substantial increase in AUC, Cmax, and volume of supply. The high plasma levels from the absorption period and increase in AUC, Cmax, and volume of supply. The percentage change of various pharmacokinetic parameters of eletriptan alone group and eletriptan with piperine combination group – single dose study.

Table 3: Comparison of pharmacokinetic parameters of Eletriptan alone group and Eletriptan with Piperine combination group – single dose study

<table>
<thead>
<tr>
<th>PK Parameters</th>
<th>Eletriptan alone</th>
<th>Eletriptan with Piperine</th>
<th>% Change after treatment with Piperine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>136.57±3.50</td>
<td>207.27±5.35***</td>
<td>51.76</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>2.25±0.17</td>
<td>2.42±0.08***</td>
<td>5.55</td>
</tr>
<tr>
<td>AUC0–24 (ng.hr/ml)</td>
<td>906.20±34.44</td>
<td>1567.18±67.25***</td>
<td>72.93</td>
</tr>
<tr>
<td>AUC0–∞ (ng.hr/ml)</td>
<td>1039.62±41.86</td>
<td>1917.36±82.85***</td>
<td>84.42</td>
</tr>
<tr>
<td>AUC%</td>
<td>12.78±0.55</td>
<td>18.24±0.59***</td>
<td>42.72</td>
</tr>
<tr>
<td>AUMC0–24 (ng/ml/h*h)</td>
<td>6872.45±285.99</td>
<td>13302.75±670.96***</td>
<td>93.56</td>
</tr>
<tr>
<td>AUMC0–∞ (ng/ml/h*h)</td>
<td>11592.72±598.09</td>
<td>26525.32±1414.24***</td>
<td>128.82</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>7.91±0.35</td>
<td>9.49±0.32**</td>
<td>19.97</td>
</tr>
<tr>
<td>MRT0–24 (hr)</td>
<td>7.58±0.13</td>
<td>8.47±0.09***</td>
<td>11.74</td>
</tr>
<tr>
<td>MRT0–∞ (hr)</td>
<td>11.14±0.27</td>
<td>13.81±0.27***</td>
<td>23.96</td>
</tr>
<tr>
<td>Clearance (L/h)</td>
<td>0.0019±0.0001</td>
<td>0.0011±0.00005***</td>
<td>-42.10</td>
</tr>
<tr>
<td>Volume of distribution (Vd) (L)</td>
<td>0.0144±0.0007</td>
<td>0.0576±0.0358 1.5</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are the Mean± S.E.M. of six rats; ***p<0.001 vs Eletriptan group, **p<0.01vs Eletriptan group, *p<0.05 vs Eletriptan group; Data is analyzed by unpaired student’s t test.
constant suggests that piperine could inhibit the hepatic removal of eletriptan. Piperine was reported to reduce the clearance of midazolam because of an inhibitory effect of piperine on CYP3A4. Pretreatment with piperine substantially improved the AUC and Cmax of eletriptan that might be a result of amplified GIT absorption of eletriptan through the blocked of both P-GP via drug out flux, and CYP3A via drug metabolism.

CONCLUSION

The progress in absorption of eletriptan could possibly be ascribed to the blocked of both P-GP and CYP3A from the gut and liver from piperine. Piperine improved the oral pharmacokinetics of eletriptan, implying that joint use of piperine and eletriptan could possibly be useful in reducing the dose of eletriptan and need close observation of potential medication interactions in patients. The combo of piperine with another CYP3A receptor show and P-GP double strand can enhance the absorption of medication that has poor oral bioavailability. Further studies are suggested to confirm their influence on people.

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Conflict of Interest

The authors affirm that they have no divergences of interest to disclose.

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