Studies on effect of co-administration of Trikatu and its components on oral bioavailability of Ampicillin and Norfloxacin, in rabbits

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**ABSTRACT**

Ampicillin and Norfloxacin are used to treat variety of Bacterial infections in Humans. These two drugs possess poor oral bioavailability as found from literatures. Ayurvedic literatures deal with the enhancement of oral bioavailability by TRIKATU (mixture of alcoholic extracts of dried fruits of *Piper longum*, dried fruits of *Piper nigrum* and dried rhizomes of *Zingiber officinalis*) or by its components. The study was aimed to find out the efficacy of TRIKATU or its components in enhancing bioavailability of the above antibiotics. The extracts of components of TRIKATU were prepared by soxhlation using alcohol (90%). The study was carried out in rabbits by oral administration of the extracts of the components and their combination in equal proportions (TRIKATU) along with above antibiotics. Blood samples were drawn at time intervals equal to three half–lives of the antibiotics and their plasma concentrations were estimated microbiologically. TRIKATU and its components enhanced the Bioavailability of Ampicillin, but the components produced no effect on Norfloxacin’s Bioavailability. Trikatu has shown maximal enhancement for both antibiotics.

**Key words:** TRIKATU, Bioavailability, Ampicillin, Norfloxacin

**INTRODUCTION**

Ayurveda means the “Science of life”; the chief source of Indian medicine, has been reused now a days instead of allopathy. This is because of large portion of Indian population are in rural areas covered under the herbal medicinal sources. When we combine ayurveda with allopathy it shows a miraculous consequence [1]. “Trikatu” the three acrids used very frequently in Ayurveda; is the combination of common culinary herblas like Black Pepper (*Piper nigrum* Linn.), Long Pepper (*Piper longum* Linn.) and Ginger (*Zingiber officinalis* Rose.). The evidence of use of these came out with report stating their effect on oral bioavailability of the drugs with which they are combined [2–4].Literatures reveal that its individual components enhance the bioavailability of many drugs [2–4]. Though Ampicillin and Norfloxacin are extensively used in treating various infections, they have problems in their oral bioavailability [5]. The oral bioavailability of Ampicillin is 62±17% and that of Norfloxacin is 30% to 40% [5, 6]. This study was aimed to find out the efficacy of “Trikatu” and its individual components in enhancing oral bioavailability of Ampicillin and Norfloxacin using animal models (Rabbits).

**MATERIALS AND METHODS**

The components of Trikatu namely, the dried fruits of Black Pepper (*Piper nigrum* Linn.), and Long Pepper (*Piper longum* Linn.) and the dried rhizomes of Ginger (*Zingiber officinalis* Rose) were procured from M/s Indian Medical Practitioners Co-operative Stores (IMCOPS), Chennai and their Pharmacognostical characters were ascertained. The Antibiotics were purchased from M/s. MMC Laboratories, Chennai and the samples were found to comply with specifications as per Indian Pharmacopoeia [7]. The test microorganism; *Bacillus subtilis* (ATCC 6633) was obtained from National Chemical Laboratories (NCL), Pune. The Culture and Assay Media were supplied by M/S Hi-Media Ltd., Mumbai. The alcoholic extracts were prepared by Soxhlation, using alcohol (90%) as solvent [2,3]. The rabbits of either sex were procured from the Animal House of Rajah...
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Muthiah Medical College, Annamalai University. The animals were maintained in a diet and quantity of water as prescribed by Central animal house, Rajah Muthiah Medical College and Hospital, Annamalai University and were housed at ambient temperature (25±1°C). The Plasma concentrations of Ampicillin and Norfloxacin were determined microbiologically (Filter paper disc – diffusion assay) using *Bacillus subtilis* (ATCC 6633) as test organism [8,9]. Peak plasma concentration (Cmax) and time to reach the peak plasma concentration (Tmax) were calculated from the actual plasma data. The Elimination rate constant (Kel) was calculated by regression analysis of the mono exponential declining line of the log plasma drug concentration versus time graph (10), while elimination half life (t1/2el) was obtained from the formula $t1/2el=0.693/Kel$. Absorption rate constant (Ka) was calculated by the method of residuals. The Area under Curve (AUC) was determined by Trapezoidal rule [11]. Extension of the AUC data to infinity (AUC∞) was done by dividing the last observed concentration of drug in plasma by Kel. Relative bioavailability (Fr) was calculated by dividing the AUC of tests (antibiotics+adjuvants) by AUC of Standard (antibiotics alone). The significance between respective treatment group was calculated by using a paired student’s ‘t’ test.

### Results and Discussion

#### Effect of Trikatu and its components on oral bioavailability of Ampicillin

As Fig.1 & Tab 1 convey that there was a definite increase in bioavailability of Ampicillin on co-administration with Trikatu and its individual components. The enhancement of bioavailability of Ampicillin was 133.803% by *Piper nigrum*, 114.24% by *Piper longum*, 122.185% by *Zingiber officinalis* and 242.571% by Trikatu. So Trikatu produced maximal bioenhancement of oral bioavailability of Ampicillin. All the data fitted to one compartment model with first order kinetics. The enhancement may be due to the action of piperine present in *Piper nigrum* and in *Piper longum* and may be due to an unknown principle in *Zingiber officinalis*. Trikatu produced an additive effect. However the enhancement of bioavailability of Ampicillin by Trikatu and its components was not significant statistically (P<0.1).

#### Effect of Trikatu and its components on oral bioavailability of Norfloxacin

As Fig-2 & Tab-2 show that there is slight increase in bioavailability with co-administration of Norfloxacin along with Trikatu (134%) whereas *P.nigrum*, and *P. longum* and *Z.officinalis* showed no increase in bioavailability of Norfloxacin. All the data fitted to one compartment model with first order kinetics. Here also the enhancement of bioavailability by Trikatu was due to additive effect. The

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**Table:**

<table>
<thead>
<tr>
<th>Rabbits Group No.</th>
<th>Drug</th>
<th>Adjuvant</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Antibiotic (150 mg/kg body weight)</td>
<td>+ No adjuvant (Control)</td>
</tr>
<tr>
<td>II</td>
<td>Antibiotic (150 mg/kg body weight)</td>
<td>+ Suspension I of <em>P. nigrum</em> (30 mg/kg body weight)</td>
</tr>
<tr>
<td>III</td>
<td>Antibiotic (150 mg/kg body weight)</td>
<td>+ Suspension II of <em>P. longum</em> (30 mg/kg body weight)</td>
</tr>
<tr>
<td>IV</td>
<td>Antibiotic (150 mg/kg body weight)</td>
<td>+ Suspension III of <em>Z. officinalis</em> (30mg/kg body weight)</td>
</tr>
<tr>
<td>V</td>
<td>Antibiotic (150 mg/kg body weight)</td>
<td>+ Suspension of Trikatu (Suspension I + II + III)</td>
</tr>
</tbody>
</table>

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bioavailability enhancement by Trikatu was not significant statistically (P<0.1).

The statistical insignificance of bioavailability enhancement by Trikatu and its components in case of Ampicillin and no enhancement by components of Trikatu and little enhancement by Trikatu in case of Norfloxacin indicate that doses of adjuvants (Piper nigrum, Piper longim and Zingiber officinalis and Trikatu) should be increased to produce a significant effect.

Table: 1  Effect of TRIKATU and its components on Oral Bioavailability of Ampicillin

<table>
<thead>
<tr>
<th>Drug combinations</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (Hrs)</th>
<th>t1/2 (Hrs)</th>
<th>(AUC)0-4.5 hrs (µg/ml/hr)</th>
<th>(AUC)α (µg/ml/hr)</th>
<th>Fr(As %)</th>
<th>Ke(Hr⁻¹)</th>
<th>Ka(Hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin alone</td>
<td>44.668± 0.27</td>
<td>1.0 ± 0.31</td>
<td>1.3 ± 0.46</td>
<td>103.712 ± 0.52</td>
<td>21.6467± 0.32</td>
<td>0.424 ± 0.47</td>
<td>1.3± 0.34</td>
<td></td>
</tr>
<tr>
<td>Ampicillin + P.nigrum</td>
<td>74.989± 0.37</td>
<td>1.5 ± 0.25</td>
<td>1.6 ± 0.22</td>
<td>137.733 ± 0.28</td>
<td>49.9268± 0.28</td>
<td>0.260 ± 0.28</td>
<td>1.4± 0.36</td>
<td></td>
</tr>
<tr>
<td>Ampicillin + P.longum</td>
<td>59.566± 0.38</td>
<td>1.0 ± 0.34</td>
<td>1.4 ± 0.23</td>
<td>118.480 ± 0.22</td>
<td>58.019± 0.24</td>
<td>0.207 ± 0.26</td>
<td>1.5± 0.28</td>
<td></td>
</tr>
<tr>
<td>Ampicillin + Z.officinalis</td>
<td>59.566± 0.38</td>
<td>1.0 ± 0.34</td>
<td>3.0 ± 0.28</td>
<td>126.721± 0.25</td>
<td>52.2145± 0.33</td>
<td>0.231 ± 0.31</td>
<td>1.4± 0.28</td>
<td></td>
</tr>
<tr>
<td>Ampicillin + TRIKATU</td>
<td>149.624± 0.25</td>
<td>1.0 ± 0.23</td>
<td>1.4 ± 0.23</td>
<td>251.576± 0.26</td>
<td>33.2975± 0.32</td>
<td>0.142 ± 0.26</td>
<td>1.97± 0.35</td>
<td></td>
</tr>
</tbody>
</table>

(Values expressed are Mean ± S.D.)*  n= 6, p< 0.05

Table: 2 Effect of TRIKATU and its components on Oral Bioavailability of Norfloxacin

<table>
<thead>
<tr>
<th>Drug combinations</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (hrs)</th>
<th>t1/2 (hrs)</th>
<th>(AUC)0-12 hrs (µg/ml/hr)</th>
<th>(AUC)α (µg/ml/hr)</th>
<th>Fr(As %)</th>
<th>Ke(Hr⁻¹)</th>
<th>Ka(Hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norfloxacin alone</td>
<td>10.593 ± 0.23</td>
<td>1.0 ± 0.32</td>
<td>1.75 ± 0.38</td>
<td>52.523 ± 0.25</td>
<td>15.7091± 0.28</td>
<td>0.124 ± 0.33</td>
<td>0.53 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin + P.nigrum</td>
<td>10.593 ± 0.34</td>
<td>1.0 ± 0.23</td>
<td>1.73 ± 0.22</td>
<td>52.5405±0.23</td>
<td>15.7385± 0.26</td>
<td>100.03± 0.32</td>
<td>124± 0.38</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin + P.longum</td>
<td>10.595 ± 0.27</td>
<td>1.0 ± 0.25</td>
<td>1.74 ± 0.25</td>
<td>52.7069 ±0.35</td>
<td>15.6282 ± 0.27</td>
<td>100.35± 0.42</td>
<td>125± 0.25</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin + Z.officinalis</td>
<td>10.597 ± 0.26</td>
<td>1.0 ± 0.37</td>
<td>1.73 ± 0.41</td>
<td>52.5846±0.32</td>
<td>15.7113 ± 0.28</td>
<td>100.11± 0.54</td>
<td>124± 0.27</td>
<td></td>
</tr>
<tr>
<td>Norfloxacin + TRIKATU</td>
<td>12.597 ± 0.33</td>
<td>2.0 ± 0.36</td>
<td>1.8 ± 0.32</td>
<td>70.336 ± 0.35</td>
<td>13.0685 ± 0.31</td>
<td>134.00± 0.66</td>
<td>0.62 ± 0.66</td>
<td></td>
</tr>
</tbody>
</table>

(Values expressed are Mean ± S.D.)*  n= 6, p< 0.05

CONCLUSION

TRIKATU the combination of three acrids used in Ayurveda increased the bioavailability of Ampicillin and Norfloxacin when co-administered. The study assumes importance in the light of reports stating that Trikatu reduced the bioavailability of Rifampicin [12, 13]. Since Ampicillin and Norfloxacin are currently used to treat many bacterial infections, their improved bioavailability through oral route can eliminate the need to
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administer them by more painful parenteral routes, thus eliminating risk of hypersensitivity. The doses of Ampicillin and Norfloxacin can be reduced, which may lead to reduction in dose related side effects and in cost of treatment of infectious diseases and improved patient compliance. However the results obtained with this study have to be confirmed by using human volunteers before making such claims.

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REFERENCES


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