ABSORPTION ENHANCEMENT STUDIES OF METFORMIN HYDROCHLORIDE BY USING TRIBULUS TERRESTRIS PLANT EXTRACT


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Abstract

The present study is to enhance the gastrointestinal absorption of poorly absorbable drugs. Metformin hydrochloride (HCl) is a BCS class III drug, having high solubility and poor absorption characters. The absorption of Metformin HCl is enhanced by using natural enhancer like Tribulus terrestris Linn (Gokhru plant) extract on intestinal absorption by everted sac technique using goat intestine. The drug was absorbed through goat intestine mainly by passive diffusion mechanism. The absorbed drug was determined by using U.V Visible Spectrophotometer at 234nm. This method is sensitive, rapid, accurate & economic. The absorption of Metformin HCl alone was less when compared to the combination of Metformin HCl with Tribulus terrestris Linn plant extract. After analyzing the results of all experiments it is found that the Tribulus terrestris Linn plant extract enhance the absorption of Metformin HCl from goat intestine and the absorption enhancement activity of the plant due to the presence of saponins in the extract.

Key words: Metformin hydrochloride, Tribulus terrestris Linn, Everted sac technique, Saponins.

Introduction

Drug absorption is defined as the process of movement of unchanged drug from the site of administration to systemic circulation1. The various important drug molecules like Metformin, Aspirin, Streptomycin, Neomycin, Barbiturates, Propranolol and Morphine were poorly absorbed from intestine, which results in low bioavailability 2, 3, 4, 5&6. The Metformin HCl belongs to BCS class III drug7. It has a characteristic property of high solubility and low permeability through intestinal mucosa, so it is necessary to increase the permeability of the drug. Orally
ingested Metformin HCl molecule absorbed into systemic circulation mainly by passive diffusion mechanism. Number of pharmaceutical chemists is working from few decades to modify physico-chemical properties of Metformin HCl to enhance its absorption by oral ingestion is the most convenient and commonly used route of drug delivery. Oral route of administration has been received more attention in the pharmaceutical field because of more flexibility in the designing of dosage form than drug delivery design for other routes. But the absorption of several drugs given by oral route can be a questionable matter. Because most of the drugs belonging to BCS III class doesn’t permeate through the intestinal epithelium and thereby rate of absorption also affected. So, it is necessary to know about absorption mechanism. A drug that is completely but slowly absorbed may fail to show therapeutic response as the plasma concentration for desired effect is never achieved. On the contrary, a rapidly absorbed drug attains the therapeutic level easily to elicit pharmacological effect. Thus, both the rate and extent of drug absorption are important.

*Tribulus terrestris* Linn (Family: Zygophyllaceae) commonly known as Gokhru is an important traditional medicinal plant of India (figure 4). Gokhru is important for its medicinal value in Ayurvedic, Siddha and Unani medicinal system. Different parts of the plant are found useful for diuretic, demulcent, anti-inflammatory, anabolic, spasmolytic, muscle relaxant, hypotensive, and hypoglycemic activity. Phytochemical study of the plant showed the presence of saponin, flavanoids and carboiline alkaloids. Therefore the present study aims to investigate effect of *Tribulus terrestris* Linn. Plant extract on intestinal absorption of Metformin HCl using everted sac technique.

![Gokhru Plant (Stem, leaves and Fruits)](image)
Materials and Methods

Chemicals and Drugs

Potassium hydroxide, Boric acid, Sodium hydroxide, ethanol, Calcium chloride, magnesium chloride, Disodium hydrogen phosphate, Sodium hydrogen phosphate, Sodium bicarbonate, Potassium chloride, Sodium chloride, Glucose were purchased from bros Scientifics, Tirupati. Metformin HCl obtained from edict pharmaceuticals, Chennai as a gift sample. All chemicals were used analytical grade only.

Plant materials and Preparation of Plant extract

Fresh fruits, leaves and stem of *Tribulus terrestris* Linn were collected from Kurnool surroundings and washed with distilled water. Collected materials were cut into the small pieces with knife and shade dried for 15 days, then crushed in a mortar mechanically to get powder. The powder material was collected and subjected to hot solvent extraction in a soxhlet apparatus by using ethanol solvent at a temperature of 70- 100˚C. Before and after every extraction the marc was completely dried and weighed. The filtrate was evaporated to dryness in a water bath.

Determination of λ max of Metformin HCl

Dissolve 100 mg of Metformin HCl in 1000 ml of phosphate buffer solution (pH 7.4) to make stock solution of 100µg/ml. From that stock solution pipette out 1ml and make up the volume up to 10 ml with buffer solution. The absorbance of this solution was scanned from 200 to 400 nm, taking phosphate buffer solution as blank. A graph was plotted by taking wavelength on X-axis and absorbance on Y-axis. Maximum absorbitivity was identified as λmax of Metformin HCl. The λmax of Metformin HCl in presence of Gokhru extract was also been identify resulting solution is made up to 2000 ml with buffer solution.

Construction of calibration curve

An accurately weighed 100 mg of Metformin was dissolved in pH 7.4 phosphate buffer as per I.P and make up the volume up to 100 ml in a volumetric flask. From this 10 ml of solution was pipette out and make up the volume up to 100 ml. Then the aliquots were prepared, whose concentration ranging from 0 to 60µg/ml and the absorbance were measured at 234 nm by using UV Spectrophotometer against the reagent blank.
Everted Sac Modification Method

To perform the absorption studies Everted-Sac modification method was selected. The experimental animal (goat) was fasted before 12-24 hrs and isolates the intestine from the animal. After isolation supply the oxygen and nutrition to the intestine. Cut the intestine into pieces and taken 2 pieces having 15cm length then the intestine is everted. After eversion the mucosal side comes out and serosal side is present inside, tied the one end of the intestine and the other end of the intestine is connected to a cannula. Take 30ml of plain buffer solution inside the intestine and the intestine is immersed in buffer solution. The pH was maintained between 8 to 8.2. The temperature was maintained at 37°C ± 0.5°C. Peristaltic movements are provided with stirrer. The buffer solution contains the drug present in outside (mucosal side) and plain buffer present in the intestine (serosal side). In this technique repeated sampling is possible.

Experimental Procedure

Place 1.5 Lt of Metformin HCl buffer solution in one organ bath and place the Metformin HCl with *Tribulus terrestris* plant extract buffer solution in another organ bath. Supply the oxygen and temperature. Provide the agitation through stirrer. Place the intestine in the organ bath with cannula only the intestine was immersed into the solution. Exact 10cm of intestine was immersed take the plain buffer into cannula

Sampling technique

5ml of sample is taken from each intestine at the interval of 15 min. The samples are pipette out for 1hr. Samples are analysed by U.V. spectrophotometer in the following manner.

Analytical method for analysis of sample

Collected samples are making up to 10ml with the water and analysed at 234 nm and determine the absorbance. The plain buffer is used as blank solution. From the absorbance calculate the concentration and amount of drug absorbed for both. The results are shown in the Table 1 & 2. A graph was plotted between the absorbed drug on Y axis and time on X axis. The resultant graph was shown in the figure 2 & 3. Compare the results for plain Metformin HCl and Metformin HCl with plant extract.
Table 1: Absorption of Metformin HCl.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time(min)</th>
<th>Absorbance</th>
<th>Concentration(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>0.8</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0.10</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>0.15</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0.20</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>0.25</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 2: Absorption of Metformin HCl with Gokhru plant Extract.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time(min)</th>
<th>Absorbance</th>
<th>Concentration(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
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<td>7</td>
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<tr>
<td>2</td>
<td>30</td>
<td>0.15</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
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<tr>
<td>4</td>
<td>60</td>
<td>0.25</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>0.32</td>
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Results and Discussion

The λ max of the Metformin HCl was found to be 234 nm showed absorbtivity of 0.326 (Figure - 2). Same study was performed for Metformin HCl in presence of Gokhru extract and was found highest absorbtivity of 0.619 at the same wavelength (Figure - 3). The study reveals that the λ max of Metformin HCl remain solution as blank. The concentration of Metformin HCl absorbed was analysed using calibration curve. Same in presence or without presence of Gokhru extract. The calibration curve of Metformin HCl found linear and thus it obeys Beers Lamberts law. Resultant graph showed in the figure 1. The effect of Gokhru plant extract on intestinal absorption of Metformin HCl is tabulated in Table 1 and Table 2. Table 1 represents the concentration of Metformin HCl absorbed without Gokhru plant extract and Table 2 represents the data of Metformin HCl concentration absorbed
The sampling is continued for 75 min and found that the concentration of Metformin HCl absorbed is more in presence of Gokhru plant extract in all case. After 15, 30, 45, 60 and 75 min the amount of absorbed Metformin HCl were 6, 8, 12, 15, 18 µg/ml respectively, where in presence of Gokhru plant extract Metformin HCl was absorbed 7, 10, 14, 19, 22 µg/ml respectively. The study clearly suggests that the Gokhru extract enhance the absorption of Metformin HCl from goat intestine. Earlier investigation showed that saponin and flavanoids is major chemical constituent of the plant. The saponin is an important phytoconstituents and important for its absorption enhancement activity. Therefore the absorption enhancement effect of Gokhru may due to presence of saponin in the extract. Though a details study required to explore the proper mechanism and identify chemicals responsible for its absorption enhancement activity.

Figure 1: Calibration curve of Metformin HCl.

![Calibration curve of Metformin HCl](image)

Figure 2: Wavelength Vs absorbance graph to determine the λmax of Metformin HCl

<table>
<thead>
<tr>
<th>No</th>
<th>Wavelength (nm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>234.00</td>
<td>0.326</td>
</tr>
</tbody>
</table>
Figure 3: Wavelength Vs absorbance graph to determine the $\lambda_{\text{max}}$ of Metformin HCl with Gokhru plant extract.

Conclusion

In the present work attempts are made to enhance the absorption of poorly absorbable drugs from intestine. The In-Vitro everted sac modification method is selected for the absorption studies. The absorption studies are conducted for poorly absorbable drug Metformin HCl in the presence of herbal drug like Gokhru plant extract. During the absorption study the samples from intestine are collected for every 15 minutes interval and upto 1hr. The absorbed drug was analyzed by using chromogenic technique. After analyzing the results of all experiments and it was concluded that the Gokhru has absorption enhancing activity of Metformin HCl.

Acknowledgement

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