HIGHLY PURIFIED FENUGREEK GUM BASED SILICA LIPID SYSTEM FOR SIMVASTATIN: IN VIVO STUDY

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ABSTRACT
The objective of current study was to investigate anti-hyperlipidemic activity of previously developed highly purified fenugreek gum based silica lipid systems for Simvastatin in poloxamer F127 induced hyperlipidemic rats. For the preparation of highly purified fenugreek gum based silica lipid system, three different grades of silica like Aerosil® 300 Pharma, Aerosil® 380 Pharma and Aeroperl® 300 Pharma were used as hydrophilic solid carriers. Highly purified fenugreek gum as emulsifier and Camplul® MCM as oil lipid phase was used. The test samples were administered orally at a dose of 1mg/kg body weight to the poloxamer F-127 induced hyperlipidemic rats and total cholesterol, triglyceride, HDL and VLDL level in the blood were checked. All the three optimized showed significant reduction (P<0.05) in comparison with standard Simvastatin. From the study it could be concluded that developed silica lipid systems has significant effect on cholesterol, VLDL, triglyceride and HDL level as compared to plain Simvastatin.

Keywords: Silica lipid system, Simvastatin, antihyperlipidemic, poloxamer F-127.

INTRODUCTION
Hypercholesterolemia and hypertriglyceridemia are the major contributing factors either, alone or together for development of coronary heart disease and the progression of atherosclerosis. [1] Coronary heart diseases (CHD) are reported to be main cause of death in western countries and Asia. The number of heart patients suffering from CHD worldwide is gradually increasing. Several methods are presently practiced to control blood cholesterol.
level includes balance of dietary fats; bile acid sequester and HMG-CoA reductase inhibitors (statins). [2]

HMG-CoA reductase is the key enzyme in the cholesterol biosynthesis pathway. Inhibition of this pathway has proven to be the most efficient therapy for the treatment of hypercholesterolemia disorder. [3] All the drugs belonging to statins category are associated with drawback of poor water solubility and low dissolution rate which resulted into poor bioavailability.

In our previous work, studies were carried to improve the water solubility and dissolution rate of antihyperlipidemic drug Simvastatin (SIM) and three different highly purified fenugreek gum (HPFG) based silica lipid systems for Simvastatin was developed with enhance solubility and dissolution rate [4]. In current study, developed formulations are investigated for their lipid lowering effect in poloxamer F-127 induced hyperlipidemia.

MATERIALS AND METHODS

Simvastatin was a gift sample from Biocon India Ltd., Mumbai, India. Capmul® MCM EP (Glyceryl Monocaprylocaprate Type I) was acquired as a gift sample from Abitec Corporation, USA. Colloidal silicon dioxide Aerosil® 300 Pharma, Aerosil® 380 Pharma and Aeroperl® 300 Pharma were supplied by Evonik Industries Mumbai, India. Highly purified fenugreek gum extracted in house. Poloxamer F-127 was obtained as a free sample from BASF, Germany.

Preparation of HPFG based silica lipid system

It involved two steps process, in brief a liquid emulsion was prepared from 0.6% HPFG as emulsifier, 10% Camul® MCM as lipid phase containing dissolved drug 1.5% ULTRA TURRAX T-18 BASIC, IKA®. (Germany) at 17500 rpm for 10 min.

The prepared emulsion was mixed with 2.5% HPFG and 7.5% silica (Aerosil® 300 Pharma, Aerosil® 380 Pharma and Aeroperl® 300 Pharma) as solid hydrophilic carriers, dried and finally sieved to get free flowing powder. Formulation compositions for all the three batches are given in Table 1. HPFG based silica lipid system prepared with Aerosil® 300 Pharma designated as Batch A, Aerosil® 380 Pharma as Batch B and Aeroperl® 300 Pharma as Batch C.
Table 1. Optimized HPFG based silica lipid system composition (All the values indicated in %w/w)

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Batch A</th>
<th>Batch B</th>
<th>Batch C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIM</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>HPFG as emulsifier</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Capmu® MCM</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HPFG as carrier</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Silica</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Purified water</td>
<td>77.9</td>
<td>77.9</td>
<td>77.9</td>
</tr>
</tbody>
</table>

Animals
Male wistar rats of weighing 180-250 g were procured from Haffkine Institute for Training, Research & Testing, Mumbai. Animal study protocol was approved by the Institutional Animals Ethics Committee IAEC/CPCSEA Mumbai, India.

Antihyperlipidemic study
Antihyperlipidemic studies were carried out and total cholesterol, triglycerides, HDL and VLDL level in the blood were checked.

Induction of hyperlipidemia
The hyperlipidemia was induced by single dose i.p. injection of 1.0 g/kg poloxamer F-127 (20% w/v). It has an ability to cause hypertriglyceridemia and hypercholesterolemia in rodents by increasing the activity of HMG-CoA reductase threefold after 24 h of administration.[5, 6, 7]

Collection of blood and experimental design
Animals were fasted overnight before starting the experiment, anesthetized and bled by retro-orbital puncture to obtain baseline values of total cholesterol in blood so that each animal served as its own control. The rats were divided into five groups, control group receiving plain water, standard group receiving plain powder Simvastatin suspension form and three test group receiving representative formulations batch 1, batch 2 and batch 3 respectively. Each group consists of six rats and maintained on a standard diet with free access to water. Treatment with plain SIM and its formulations was started orally (1mg/kg) after 24h injection of 1.0 g/kg of (20% w/v) poloxamer F-127 and continued for 4 days. The blood was withdrawn after 24h, 48 h and at the end of four days of induction of hyperlipidemia.
Estimation of blood cholesterol, triglycerides, HDL and VLDL levels were carried out at Haffkine Institute for Training, Research and Testing, Mumbai.

**Statistical analysis**
All results are expressed as the mean±SD. The results were analyzed for statistical significance treated by one way ANOVA test (GraphPad PRISM® software version 6.01) and results with P<0.05 was considered significant.

**RESULTS AND DISCUSSION**

**Effect on Total cholesterol level (TC)**
Efficacy of Simvastatin is related to its total cholesterol lowering and increasing in high density lipoprotein (HDL) activity. Poloxamer F-127 causes 5.5 folds increase in total cholesterol level in control and plain drug treated group whereas HPFG based silica lipid based silica lipid system treated groups showed 4-4.5 folds increase after 24 h of injection. Batch1, batch 2 and batch 3 treated group showed significant decrease (P<0.05) in TC level as compare to control and plain drug treated group as illustrated in Fig. 1. Batch 2 treated group showed significant reduction TC level (240 mg/dl) with reference to control group (497 mg/dl), plain drug (440 mg/dl), batch1 (341 mg/dl) and batch 3 (322 mg/dl) treated group after 48 h of treatment. Explanation was that batch 2 contains Aerosil® 380 Pharma which posses' higher surface area (380m²/g) as compared to other used two silica and this high surface area contributed to high water solubility and dissolution rate which resulted into improved bioavailability. At the end of four days treatment, the TC levels of all silica lipid systems treated group reduced to near baseline values except control and plain drug treated groups. These activities of developed Simvastatin formulations could be correlated with improve water solubility and dissolution rate as compared to plain Simvastatin suspension.

![Graph showing total cholesterol levels](image)

**Figure 1. Total cholesterol levels in control group rats and rats after treatment with Simvastatin and its silica lipid systems. Total cholesterol values (mg/dl) are means ± SD of six animals.**
Effect on HDL level
Elevated HDL level is good for health. In this study, control group, plain SIM treated group shows 53 mg/dl and 57 mg/dl HDL level respectively after 24 h of injection Fig.2. Increase in HDL level was found with silica lipid systems treated group batch1 64 mg/dl, batch 2 70 mg/dl and batch 3 63 mg/dl. HDL level was decreases to base level after four days of treatment except silica lipid system treated group.

Figure 2. High-density lipoprotein levels in control group rats and rats after treatment with Simvastatin and its silica lipid systems. High-density lipoprotein values (mg/dl) are means ± SD of six animals.

Effect on Triglyceride level (TG)
Triglyceride level was increased significantly by 6-7 folds in all animal groups after 24 h of poloxamer F-127 injection Fig.3. In control group, TG level increased from 185 mg/dl to 1430 mg/dl. After four days of treatment, batch 1, batch 2 and batch 3 significantly reduces the elevated TG level to 187 mg/dl, 155 mg/dl and 150 mg/dl respectively as compared to control group 433mg/dl and plain SIM suspension treated group 235 mg/dl.

Figure3. Triglyceride levels in control group rats and rats after treatment with Simvastatin and its silica lipid system. Triglyceride values (mg/dl) are means ± SD of six animals.
Effect on VLDL level

Fig.4, VLDL level was increased significantly from normal value 37 mg/dl to 286 mg/dl in control group after 24 h of induction. Batch1, batch 2 and batch 3 reduced the elevated VLDL level to 37.4 mg/dl, 31.25 mg/dl and 30.27 mg/dl respectively in comparison to standard drug (SIM) to 47 mg/dl. All the results were statistically significant (P<0.05) and compared with normal and control group.

![Graph showing VLDL levels](image)

Figure 4. Very low density lipoprotein (VLDL) levels in control group rats and rats after treatment with Simvastatin and its silica lipid system. Triglyceride values (mg/dl) are means ± SD of six animals.

CONCLUSION

From the study it could be concluded that developed HPFG based silica lipid systems for Simvastatin not only have solubility and dissolution rate enhancing property but also have excellent antihyperlipidemic activity as compared to plain drug. Developed formulations significantly reduce the total cholesterol, triglyceride, VLDL and increase the HDL level.

REFERENCES


