RESEARCH ARTICLE

Evaluation of antihyperlipidemic activity of gugulipid alone and in combination with different dosage of atorvastatin in triton-induced hyperlipidemic rodent model: Exploring a possible synergistic activity

Rajiv Jash¹, Chiranjib Bagchi², Achintya Mitra³, Santanu Kumar Tripathi⁴

¹Department of Pharmacy, Sanaka Educational Trusts Group of Institutions, Durgapur, West Bengal, India, ²Department of Clinical and Experimental Pharmacology, School of Tropical Medicine, Kolkata, West Bengal, India, ³Department of Clinical Research, Central Ayurveda Research Institute, Kolkata, West Bengal, India, ⁴Department of Pharmacology, Netaji Subhas Medical College and Hospital, Bihta, Patna, Bihar, India

Correspondence to: Chiranjib Bagchi, E-mail: bchiranjib@yahoo.co.in

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ABSTRACT

Background: Gugulipid obtained from Commiphora mukul carries a long history of safe and efficacious use in hyperlipidemia as per Ayurvedic literature. Statins like atorvastatin are a highly prescribed hypolipidemic drug but not free from potentially serious adverse effects. Aims and Objectives: The present study was designed to establish antihyperlipidemic activity of gugulipid in triton-induced hyperlipidemic rats in comparison to atorvastatin and simultaneously to explore the combination of gugulipid and atorvastatin for any synergistic activity. Materials and Methods: Male Wistar albino rats (20) were divided equally into vehicle (2% gum acacia) (Group I), gugulipid only 6.75 mg/kgbw (Group II), atorvastatin 7.2 mg/kgbw only (Group III), and gugulipid 6.75 mg/kgbw and atorvastatin in 7.2 mg/kgbw combination (Group IV) in Phase 1 study. In Phase 2, additional three groups were created with five rats in each receiving gugulipid 6.75 mg/kgbw with atorvastatin at 5.4 mg/kgbw, 3.6 mg/kgbw, and 1.8 mg/kgbw dosage, respectively (Groups V–VII). Hyperlipidemia was induced by single intraperitoneal injection (400 mg/kgbw) of triton after 7 days of feeding with respective agents dissolved in vehicle through oral route. Results: Regarding total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL), Gr II was found superior to Gr I but inferior to others (P < 0.01). Gr IV prevented the rise of TC and TG significantly in comparison to Gr V, VI, and VII (P < 0.01) whereas Groups V and VI having non-significant difference in between, both differed significantly (P < 0.01) with Gr VII. Groups IV, V, and VI prevented the rise of serum LDL significantly (P < 0.01) from Group VII. Conclusion: Gugulipid showed significant antihyperlipidemic activity and was found to be optimally efficacious and safe in combination with even reduced dose of atorvastatin.

KEY WORDS: Hypolipidemic; Antihyperlipidemic; Triton; Hyperlipidemic; Gugulipid; Atorvastatin; Drug Synergism

INTRODUCTION

Hyperlipidemia is highly predictive risk factor for atherosclerosis, coronary artery disease, and cerebral vascular diseases.[¹] Atherosclerosis of arteries is a generalized disease of the arterial network known as progressive disease and silent killer characterized by the formation of lesions called atherosclerosis plaques in the walls of large and/or medium-sized coronary arteries which reduce blood flow to the myocardium called coronary artery disease.[²] Hyperlipidemia is not only secondary metabolic dysregulation associated with diabetes but also represent increased risk factor for the development of diabetes.[³-⁵] Plants are important sources of medicinal compounds and more than 70% of population of developing countries is dependent on traditional medicine
therapies for their ailments. In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice in hyperlipidemia. In the present study, the selection of gugulipid is based on the back up evidence that this natural cholesterol lowering agent, a oleo resin obtained from the plant Commiphora mukul belonging to the family of Burseraceae, has been used in Ayurvedic medicine practice for more than 2000 years to treat a variety of ailments such as rheumatism, arthritis, hyperlipidemia, obesity, and atherosclerosis. Gum resin resides in the ducts located in the soft bark of the tree and with a circular incision made on the bark stem, a pale yellow aromatic fluid exudates that quickly solidifies to a golden or reddish-brown agglomerate from which Z and E gugulsterones have been isolated as active agents possessing lipid lowering activity. It is likely that this effect accounts for the hypolipidemic activity of these phytosteroids.

Moreover, HMG Co-A reductase inhibitors (statins) are the mostly used drug class in the treatment of hyperlipidemia but there is a possibility of some serious adverse events such as rhabdomyolysis, proximal myopathy, and hepatotoxicity. Incidence of these adverse effects increases with respect to the duration of treatment and the dose. Atorvastatin is used in varying doses in humans (from 10 to 80 mg), needless to say more is the dose, more the chances of having adverse effects. The striking feature of gugulipid is, it is remarkably free from serious adverse effects. Apart from headache, diarrhea, and skin rash, it is devoid of any adverse effects on hepatic, renal function, or in hematological parameters when administered at a dose of 400 mg/day for 4 weeks.

The present study was aimed to establish the lipid-lowering activity of gugulipid in triton-induced hyperlipidemic rats in comparison to atorvastatin. At the same time, we explored the combination of gugulipid and atorvastatin for any possible synergistic effect and to find out the optimum dose of the individual agents.

MATERIALS AND METHODS

The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India. It was conducted after getting requisite approval letter from the Institutional Animal Ethics Committee.

Procurement of Study Animals, Plant Material, and Standard Drug

Male Wistar albino rats of same age group, and body weight 150–200 g, procured from a recognized laboratory animal breeder, in Kolkata. Gugulipid extract (processed guggul) was obtained from Central Ayurveda Research Institute (Erstwhile National Research Institute of Ayurvedic Drug Development), Kolkata, after getting verified by the pharmacognosy department of the institute. A single brand and batch number of the standard drug (atorvastatin) were chosen. Before recruitment in the study, all the rats were acclimatized to the laboratory environment for 1 week.

Preparation and Administration Protocol of Drugs and Chemicals

Process of extraction of gugulipid: The dry guggul resin had been processed at the pharmacognosy laboratory of Central Ayurveda Research Institute, Kolkata, following the traditional Ayurvedic literature. The dry exudate of guggul was cleaned of foreign matters, impurities and made into small pieces which were mixed with the decoction of Triphala Churna (Embelica officinalis, Terminalia chebula, and Terminalia belerica 1:1:1 w/w). Then, the mixture was boiled, manually stirred (5 h for 5 consecutive days) which turned it into a semisolid matter. Then, another decoction of gulancha (Tinospora cordifolia) was added (1/4th of the matter) and again boiled for 5 h. The semisolid mater was sieved by the cotton cloth 3 times followed by drying resulting into the processed guggul.

The gugulipid extract (processed guggul) appeared as a brown lump because of its hygroscopic nature. It was crushed with mortar and pestle to a fine powder after sieving with a fine mesh (Sieve 80). The bodyweight adjusted calculated total dose of gugulipid powder was added to freshly prepared 2% gum acacia solution and stirred continuously to make a homogenous suspension. Atorvastatin suspension was also prepared similarly. These suspensions were fed orally to the respective groups of rats using rat feeding cannula during the experiment. The Wistar albino rats in the combination treatment groups in both phases of the study were fed with calculated dose of gugulipid first followed by the atorvastatin suspension after a gap of 1 h considering the limited capacity of rat stomach.

The dose of gugulipid was selected according to the human dose, that is, 75 mg/day. This human dose was converted to rat dose by multiplying human dose with conversion factor 0.018 for 200 g rats. Hence, human equivalent dose of gugulipid came to 6.75 mg/kg bodyweight of rats.

Similarly, the maximum human dose of atorvastatin (80 mg/day) was converted to the equivalent rat dose at 7.2 mg/kg body weight.
Tyloxapol or triton (WR1339) is a non-ionic liquid polymer of the alkyl aryl polyether alcohol type, when injected intraperitoneally, blocks plasma lipolytic activity, and thus leads to breakdown of triglyceride (TG)-rich lipoproteins. Because of high specific gravity, triton solution was diluted with 0.9% sodium chloride and then it was given intraperitoneally after an overnight fast only once, at a dose of 400 mg/kgbw of each rat to make it hyperlipidemic.[13]

**Experimental Set Up**

Rats were housed in polypropylene cages, fed with pellet diet, water *ad libitum*, maintaining 12 h each light dark cycle at an ambient temperature of 25–30°C and 45–55% relative humidity.

**1st Phase of the Study**

A total of 20 rats were divided into four groups, each comprising five rats. The groups were

1. Group I – the rats received 2% gum acacia suspension
2. Group II – the rats received gugulipid at a dose of 6.75 mg/kgbw in suspension with 2% gum acacia
3. Group III – the rats received standard drug atorvastatin at a dose of 7.2 mg/kgbw in gum acacia (2%) suspension
4. Group IV – the rats received both gugulipid (6.75 mg/kg) and atorvastatin (7.2 mg/kg) simultaneously one after another at an interval of 1 h. All the rats in above groups were fed with normal rat pellet diet.

**2nd Phase of the Study**

Wister albino rats were divided into the following five groups and each group containing five rats. As Group I and Group IV were common to both Phase 1 and Phase 2, these groups were not recreated to minimize the unnecessary use of rats, so the data were carried forward to Phase 2 study, only Groups V, VI, and VII had been newly formed. Finally, the Phase 2 study constituted as follows: Group I (vehicle control): Received 2% gum acacia and also triton on the 7th day, Group IV – Group VII received 6.75 mg/kgbw of gugulipid along with the specified dose of atorvastatin suspended in gum acacia (2%) as follows: Group IV: 7.2 mg/kgbw of atorvastatin, Group V: 5.4 mg/kgbw of atorvastatin, Group VI: 3.6 mg/kgbw of atorvastatin, and Group VII: 1.8 mg/kgbw of atorvastatin.

Atorvastatin dose had been selected among rats between Gr IV and Gr VII corresponding to human equivalent dose of 80 mg, 60 mg, 40 mg, and 20 mg/day.

All the rats of four groups in Phase 1 and five groups in Phase 2 were administered triton solution intraperitoneally. Before and 24 h after triton administration, blood was collected from the rats by tail vein incision after an overnight fast and biochemically estimated.

**Biochemical Estimations**

The collected blood samples were centrifuged at 1500 rpm for 15 min for the separation of serum, which were used for lipid profile estimation.

The estimation of total cholesterol (TC), TGs, and high-density lipoprotein (HDL) was performed by semi-autoanalyzer using enzymatic reagent kits,[14] whereas estimation of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol was done using Friedewald’s[15] formula.

**Statistical Analysis**

All data were checked for completeness and statistically analyzed. Descriptive data were represented as mean and standard deviation. Different levels were expressed at 95% confidence interval and $P < 0.05$ was considered statistically significant. Mean values of same group before and after triton administration (paired situation) were compared using Wilcoxon’s matched pairs signed-rank test. Mean values of different groups were compared using Kruskal–Wallis ANOVA followed by Mann–Whitney U-test after prior analysis of data for distribution (Kolmogorov–Smirnov test). All statistical analyses were performed using statistical software packages such as Statistical Package for the Social Sciences (Windows version 11.5.; SPSS Inc., Chicago [IL], USA) and Microsoft Excel.

**RESULTS**

**Phase 1 Study**

Administration of triton WR 1339 caused significant increase in the serum lipid parameters of all the rats (Wilcoxon’s matched pairs signed-rank test). The levels of serum TC, TG, HDL, LDL, and VLDL in Groups I, II, III, and IV before and after triton administration are shown in Table 1.

Rise in TC, TG, LDL, VLDL, and HDL was highest in Group I, but among the treatment groups, Group IV reduced the rise of TC, TG, LDL, and VLDL more significantly than treatment Group II and Group III ($P < 0.01$)

Group II reduces the rise of HDL significantly in comparison to Group I ($P < 0.05$), but Group III and Group IV did not show any significant difference in comparison to Group I.

HDL was significantly raised in Group III and Group IV in comparison to Group II ($P < 0.01$).
On pairwise comparison with values before and after triton administration, all the lipid parameters raised significantly after triton administration (Wilcoxon's matched pair signed-rank test). When all four treatment groups along with vehicle control (Gr I, IV, V, VI, and VII) were compared, a significant difference of all lipid parameters was noted (TC: $P < 0.005$, TG: $P < 0.001$, HDL: $P < 0.01$, LDL: $P < 0.001$, and VLDL: $P < 0.001$) (Kruskal–Wallis ANOVA test).

However, between-group comparison (by Mann–Whitney U-test), Group IV prevented the rise of TC and TG significantly in comparison to Groups V, VI, and VII whereas though Groups V and VI had non-significant difference in between, both differed significantly with Group VII. All other three treatment groups (IV, V, and VI) prevented the rise of serum LDL significantly from Group VII [Table 2]. Group VII had significant rise of HDL in comparison to Groups I, IV, V, and VI.

**DISUSSION**

Hyperlipidemia and hypercholesterolemia are reportedly the major risk factors in lifestyle-related diseases such as atherosclerosis and related cardiovascular complications including cerebral stroke and myocardial infarction.\[^{12,16}\] Prevention or treatment of such disorders can be achieved by targeting the hyperlipidemia and hypercholesterolemia through diet and/or drug administration.\[^{16}\] In the current study, Wister albino rats were made hyperlipidemic after administration of triton (WR1339) or tyloxapol to test the antihyperlipidemic activity of gugulipid extract. Such model had been used earlier successfully for the induction of acute hyperlipidemia\[^{13}\] and for the testing of the standard and potential agents for hyperlipidemia as well.\[^{17-19}\] In Phase 1 of the present study, significant antihyperlipidemic activity was observed in only gugulipid group as compared to the vehicle only group [Table 1]. Atorvastatin only group appeared more potent in preventing the rise in TC, TG, LDL, and VLDL as compared to the gugulipid only group. However, atorvastatin and gugulipid combination group was more potent in preventing the elevation of the lipid parameters as compared to atorvastatin or gugulipid alone excepting HDL which was significantly high in comparison to gugulipid alone. Hence, it can be said that the combination has a possible synergistic effect in controlling the hyperlipidemia and thus may have a role in the clinical use. Considering the fact that chronic use of atorvastatin in higher dose has some adverse effects such as rhabdomyolysis, proximal myopathy, and hepatotoxicity, the possible synergistic effect can help us to reduce the dose of atorvastatin without compromising the therapeutic effect which was evidenced in the Phase 2 part of the study. In that phase, we have studied this combination with different dose level of atorvastatin with

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated Gr I</td>
<td>Before triton</td>
<td>61±0.89</td>
<td>22±2.4</td>
<td>18±0.54</td>
<td>38±2.89</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>180±3.75</td>
<td>67±1.75</td>
<td>30±4.47</td>
<td>135±3.72</td>
</tr>
<tr>
<td>Gugulipid treatment Gr II</td>
<td>Before triton</td>
<td>58±1.7</td>
<td>22±1.15</td>
<td>17±1.15</td>
<td>37±1.92</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>131±2.1</td>
<td>55±2.83*</td>
<td>25±1.17**</td>
<td>94±3.68*</td>
</tr>
<tr>
<td>Atorvastatin treatment Gr III</td>
<td>Before triton</td>
<td>45±2.26</td>
<td>16±2.21</td>
<td>18±1.18</td>
<td>23±2.23*</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>112±4.06*</td>
<td>40±1.2*</td>
<td>34±1.89</td>
<td>69±4.25*</td>
</tr>
<tr>
<td>Atorvastatin (7.2 mg/kg)+ Gugulipid Gr IV</td>
<td>Before triton</td>
<td>33±3.58</td>
<td>15±0.5</td>
<td>14±1.10</td>
<td>15±1.97</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>91±1.69*</td>
<td>31±2.63*</td>
<td>31±0.86</td>
<td>54±1.16*</td>
</tr>
</tbody>
</table>

* $P < 0.01$ regarding TC in Gr IV comparison to Gr II and III and in Gr III corresponding to Gr II, regarding TG in Gr IV in comparison to Gr III, in Gr III and IV comparison to Gr I and II, in Gr II comparison to Gr I, regarding HDL in Gr II comparison to Gr III and IV, regarding LDL in Gr II, III, and IV comparison to Gr I, in Gr III and IV comparison to Gr II, in Gr IV comparison to Gr III. $P < 0.05$ regarding HDL in Gr II comparison to Gr I, regarding LDL in Gr IV comparison to Gr III (Mann–Whitney U-test). LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein.
a standard dose of gugulipid to find out the optimally effective and safe dose regimen which was not explored earlier. Atorvastatin 7.2 mg/kgbw and gugulipid 6.75 mg/kgbw in combination appeared superior in preventing the rise of TC and TG, whereas atorvastatin 3.6 mg/kgbw and gugulipid 6.75 mg/kgbw appeared as an optimally effective acceptable combination to start with, in preventing the rise of TC, TG, and LDL also catalyzing the rise of HDL with reduced chance of statin-induced toxicity. In this short duration study, the rats had normal eating and drinking habits with no significant changes in their bodyweights and without any mortality which demands a long-term toxicity study.

The Phase 1 study result was in consistence with another published study from the same research group in rabbit model where atorvastatin and gugulipid combination was seen to possess comparable hypolipidemic activity in comparison to atorvastatin or gugulipid monotherapy.\[20\]

As per available literature, gugulipid appears to be as a safe hypolipidemic agent that possesses an immense potential to be combined with a reduced dose of atorvastatin for long-term therapeutic use in humans.\[28\] In the present study, as a monotherapy, gugulipid did not appear a superior alternative to rodent equivalent highest recommended human dose of atorvastatin. However, it proved to have immense potential to be combined with a reduced dose of atorvastatin and still possessing a substantial antihyperlipidemic property with a less chance of toxicity. The two different mechanism of actions of these agents are fulfilling the principle of drug synergism when used in combination and thus worth trying.

### Table 2: Result of Phase 2 of the study

<table>
<thead>
<tr>
<th>Treatment groups n=5</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated (Group I)</td>
<td>Before triton</td>
<td>61±0.89</td>
<td>22±2.4</td>
<td>18±0.54</td>
<td>38±2.89</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>180±3.75</td>
<td>67±1.75</td>
<td>30±4.47</td>
<td>135±3.72</td>
</tr>
<tr>
<td>Atorvastatin (7.2 mg/kg)+Gugulipid (Group IV)</td>
<td>Before triton</td>
<td>33±3.58</td>
<td>15±0.5</td>
<td>14±1.10</td>
<td>15±1.97</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>91±1.69°</td>
<td>31±2.63°</td>
<td>31±0.86</td>
<td>54±1.16</td>
</tr>
<tr>
<td>Atorvastatin (5.4 mg/kg)+Gugulipid (Group V)</td>
<td>Before triton</td>
<td>34±2.83</td>
<td>19±2.01</td>
<td>14±1.81</td>
<td>17±0.62</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>100±0.89°</td>
<td>42±2.93°</td>
<td>33±1.11</td>
<td>53±3.69</td>
</tr>
<tr>
<td>Atorvastatin (3.6 mg/kg) + Gugulipid (Group VI)</td>
<td>Before triton</td>
<td>36±1.61</td>
<td>19±1.52</td>
<td>13±1.81</td>
<td>18±2.09</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>103±1.36°</td>
<td>41±0.89°</td>
<td>32±2.16</td>
<td>53±2.16</td>
</tr>
<tr>
<td>Atorvastatin (1.8 mg/kg) + Gugulipid (Group VII)</td>
<td>Before triton</td>
<td>44±0.83</td>
<td>21±3.58</td>
<td>15±1.19</td>
<td>21±1.89</td>
</tr>
<tr>
<td></td>
<td>After triton</td>
<td>136±3.68</td>
<td>64±3.16</td>
<td>44±0.79°</td>
<td>70±4.28</td>
</tr>
</tbody>
</table>

*P < 0.01 regarding TC and TG in Gr IV in comparison to Gr V, VI, VII and Gr V and VI in comparison to Gr VII, regarding TG in Gr IV, V, and VI in comparison to Gr I and regarding HDL in Gr VII in comparison to Gr I, Gr IV, V, and VI, regarding LDL in Gr IV, V, VI, and VII in comparison to Gr I, in Gr IV, V, and VI comparison to Gr VII, regarding VLDL in Gr IV, V, and VI in comparison to Gr I, in Gr V and VI comparison to Gr VII (Mann–Whitney U-test). LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein.

Thus, the result of the present study necessitates further pharmacokinetic and pharmacodynamic exploration for any possible interaction between atorvastatin and gugulipid when used in combination. A number of clinical trials had been conducted using gugulipid with majority showing favorable results in reducing TC, TG, and a few with not so impressive effect.\[22,25,10\] In those studies, gugulipid was used as a monotherapy versus placebo in majority or clofibrate in one and none with statin. However, in overall pooled analysis of four studies, gugulipid was found to have a comparable magnitude of hypolipidemic effect similar to the available drugs in modern medicine.\[10\] Noteworthy, though of short duration, those studies documented...
favorable safety profile of gugulipid with a low incidence of dyspepsia and mild skin rash.

Our study was short duration and was primarily focused on finding out the efficacy and overall safety of the gugulipid and atorvastatin combination in rodent model to find out an optimum combination dose. We did not perform a detailed toxicity study of gugulipid nor study the pharmacokinetic characteristics and mechanism of pharmacokinetic and pharmacodynamic interaction with atorvastatin if any. This was a limitation of the study. We only observed a possible pharmacodynamic drug synergism between gugulipid and atorvastatin with different dosage schedule which was not done earlier. This preliminary study was successful to draw the hypothesis confidently through experimental rodent model. This is the real strength of the present study. Nevertheless, there is a definite scope to explore this combination for the proposed synergistic activity through an in-depth pharmacokinetic and mechanistic experimental study. In addition, considering the high efficacy but potential adverse effect of statins, a well-designed clinical safety and efficacy study with different dosage regimen of atorvastatin or any other statin in combination with gugulipid is the need of the hour.

CONCLUSION

In the present study, a synergistic effect of atorvastatin and gugulipid combination in the prevention of triton induced hyperlipidemia pointed toward the scope of reduction of atorvastatin dose and associated toxicity. Atorvastatin (3.6 mg/kg bw) and gugulipid (6.75 mg/kg bw) appeared as an optimally efficacious and safe combination to start with in hyperlipidemia as evidenced in rodent model which warrants extensive pharmacokinetic and toxicity analysis along with confirmatory clinical efficacy and safety study to establish fruitfulness of this combination.

REFERENCES


Source of Support: Nil, Conflicts of Interest: None declared.