An Investigation into Anti-Dyslipidemic Activity of Isovaleric Acid in Wistar Rats Fed Fructose-Rich High Fat Diet

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Abstract
Introduction: Correction of dyslipidemias is important for prevention of atherosclerotic diseases. Limitations of the currently available anti-dyslipidemic drugs prompted us to investigate the anti-dyslipidemic activity of isovaleric acid isolated from Valeriana wallichii.

Methods: The study was conducted on 24 healthy male adult Wistar rats (200-225 gm) divided into four groups of six each. Group I was kept on normal diet (Hindustan Lever Food Pellets). Group II was fed fructose-rich high fat diet (F-HFD). Group III was given F-HFD+isovaleric acid (25 mg/kg of body weight/day), and group IV was given F-HFD+atorvastatin (10 mg/kg body weight/day). After 30 days, plasma lipids and lipoproteins were measured in all the rats.

Results: Feeding F-HFD resulted in significant dyslipidemia. Isovaleric acid and atorvastatin were equally effective in preventing the F-HFD-induced dyslipidemia.

Conclusions: Further studies are warranted to realize the anti-dyslipidemic potential of isovaleric acid in animals and humans.

Keywords: Dyslipidemia; isovaleric acid; atorvastatin.

Introduction
Dyslipidemia usually comprises an increase in serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C), and a decrease in high density lipoprotein cholesterol (HDL-C), either singly or in combination.¹ Long term dyslipidemia results in vascular changes and dysfunction.² Therefore, dyslipidemia is recognized as a risk factor in the initiation and development of atherosclerosis and cardiovascular diseases.³,⁴ It has also been shown...
that controlling dyslipidemia for long periods with the currently available anti-dyslipidemic drugs is not easy.\textsuperscript{3,4,5} Therefore, there is a need to develop safe and effective anti-dyslipidemic agents. Isovaleric acid is otherwise known as 3-methylbutanoic acid.\textsuperscript{6} It is usually added as a flavoring agent to wine.\textsuperscript{6} It has been proposed that it is an anticonvulsant.\textsuperscript{7} Isovaleric acid is a component of \textit{Valeriana wallichi}, a plant that we are investigating as a possible anti-dyslipidemic therapy in animals. In view of the encouraging results obtained so far (unpublished observations), we decided to investigate whether the anti-dyslipidemic activity of \textit{Valeriana wallichi} might be due to isovaleric acid present in it. Atorvastatin was used as a reference drug for comparison.

**Material and Methods**

The study was conducted on 24 male Wistar rats (200-225 gm) after receiving approval from the institutional ethics committee of King George’s Medical University, Lucknow. The rats were divided into four groups of six each. Initially, all the groups were kept on Hindustan Lever Food Pellets and water ad libitum. They were put in cages and kept in a temperature and humidity controlled room with a 12-hour light-dark cycle. After acclimatization, group I (normal diet control) continued to receive normal pellet diet. Group II was switched to fructose-rich high fat diet (F-HFD) to induce dyslipidemia. F-HFD (1 kg) was prepared by mixing 610 gm of normal pellet diet, 280 gm of ground nut oil, 5 gm of cholesterol, 5 gm of deoxycholic acid and 100 gm of fructose. Group III was given F-HFD and oral isovaleric acid (25 mg/kg of body weight/day) isolated from \textit{V. wallichi}. Group IV was given F-HFD and oral atorvastatin (10 mg/kg of body weight/day). At the end of experimental period (30 days), blood samples were collected from retro-orbital plexus of each rat in EDTA tubes, and plasma was separated by centrifugation. Plasma cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were measured by CHOD-PAP, GPO-POD, PEG-PAP and immunoturbidimetric methods.\textsuperscript{8,9,10,11,12} Very low density lipoprotein cholesterol (VLDL-C) was calculated by Friedewald formula.\textsuperscript{13} Estimation of free fatty acids (FFA) was done by the method of Monsinger et al.\textsuperscript{14} Phospholipids in plasma were determined by Malachite green method.\textsuperscript{15} Plasma lecithin cholesterol acyltransferase (LCAT) activity was measured by the method of Albers et al.\textsuperscript{16} Wind and Robinson method was used to measure post heparin lipolytic activity (PHLA).\textsuperscript{17}

**Statistical Analysis**

InStat3 package was used for statistical analysis. The mean± SD of different groups were compared by using one way analysis of variance (ANOVA), followed by Student-Newman-Keuls test. P<0.05 was considered significant.

**Results**

Table 1 shows that plasma TC, TG, LDL-C, VLDL-C and HDL-C of group II were significantly higher as compared to group I. This shows that feeding F-HFD for 30 days induced significant dyslipidemia. Plasma TC, TG, LDL-C, VLDL-C and HDL-C of group III and IV were significantly lower as compared to group II. This shows that administration of isovaleric acid or atorvastatin together with F-HFD prevented dyslipidemia. No significant difference was seen in the plasma TC, TG, LDL-C, VLDL-C and HDL-C of group III and group IV. This means that the anti-dyslipidemic effect of isovaleric acid was comparable to that of atorvastatin.
The aim of the present study was to explore the anti-dyslipidemic activity of a compound isolated from the extract of *V. wallichi*, namely isovaleric acid. Atorvastatin was used as a reference drug for comparison. Dyslipidemia was induced in rats by feeding them fructose rich-high fat diet (F-HFD). Our results show that: (i) feeding F-HFD resulted in significant dyslipidemia, (ii) both isovaleric acid and atorvastatin were able to prevent dyslipidemia caused by F-HFD and (iii) the anti-dyslipidemic effects of atorvastatin and isovaleric acid were comparable. Feeding F-HFD also increased plasma FFA but had no effect on plasma phospholipids, LCAT and PHLA. In the group given isovaleric acid together with F-HFD, plasma FFA, phospholipids and PHLA were significantly lower and LCAT significantly higher as compared to the group given F-HFD alone. Similar results were obtained in the group given atorvastatin together with F-HFD. The effects of atorvastatin and isovaleric acid were comparable. Thus, isovaleric acid emerges as a potential anti-dyslipidemic agent having an efficacy similar to that of atorvastatin, an established anti-dyslipidemic agent having an efficacy similar to that of atorvastatin, an established anti-dyplipidemic drug. We have not gone into the mechanism of action of isovaleric acid but the change in plasma LCAT activity seems to provide a clue. Studies have revealed a possible association between plasma cholesterol level and LCAT activity. Extrahepatic cholesterol is transported to liver by HDL. For this, cholesterol present in other lipoproteins has to be transferred to HDL. This transfer takes place with the help of

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Group I (Normal diet, n=6)</th>
<th>Group II (F-HFD, n=6)</th>
<th>Group III (F-HFD + isovaleric acid, n=6)</th>
<th>Group IV (F-HFD + atorvastatin, n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>62.6±9.72</td>
<td>128±18.2a</td>
<td>67.3±9.70b</td>
<td>68.3±11.6c</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>48.1±11.2</td>
<td>110±16.6a</td>
<td>58.6±10b</td>
<td>57±4.73d</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>28.3±8.61</td>
<td>40.5±6.86e</td>
<td>29.3±6.25e</td>
<td>29.1±6.79e</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>24.6±19.2</td>
<td>65.5±19.2a</td>
<td>26.2±11.3e</td>
<td>27.7±12.3e</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>9.6±2.2</td>
<td>22±3.3a</td>
<td>11.7±2.1c</td>
<td>11.4±0.94c</td>
</tr>
</tbody>
</table>

*P<0.001 when compared with group I; ^P<0.05 when compared with group I
^P<0.001 when compared with group II; ¥P<0.05 when compared with group II

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Group I (Normal diet, n=6)</th>
<th>Group II (F-HFD, n=6)</th>
<th>Group III (F-HFD + isovaleric acid, n=6)</th>
<th>Group IV (F-HFD + atorvastatin, n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA (mg/dl)</td>
<td>62.5±18.9</td>
<td>90.1±11.6e</td>
<td>59.8±12.2e</td>
<td>59.1±17.1e</td>
</tr>
<tr>
<td>PL (mg/dl)</td>
<td>102.3±23.7</td>
<td>128±26.3x</td>
<td>81±6.48x</td>
<td>80.6±3.14x</td>
</tr>
<tr>
<td>LCAT (nmol/ml/h)</td>
<td>36.3±14.7</td>
<td>44.1±13.4e</td>
<td>57.8±10y</td>
<td>59.8±10.4y</td>
</tr>
<tr>
<td>PHLA (nmol/ml/h)</td>
<td>21±3.8</td>
<td>21.6±5.5x</td>
<td>12.8±2.63y</td>
<td>11.8±2.63y</td>
</tr>
</tbody>
</table>

*P<0.05 when compared with group I; ^P> 0.05 when compared with group I
¥P< 0.01 when compared with group II; ¥P< 0.05 when compared with group II

### Discussion

Studies have revealed a possible association between plasma cholesterol level and LCAT activity. Extrahepatic cholesterol is transported to liver by HDL. For this, cholesterol present in other lipoproteins has to be transferred to HDL. This transfer takes place with the help of
Thus, increased LCAT activity might be one of the reasons for the anti-dyslipidemic effect of isovaleric acid. There might be other reasons too.

One limitation of the present study was its small sample size. Secondly, it is to be seen whether the results obtained in Wistar rats can be replicated in human beings. Safety of isovaleric acid also needs to be established. Yet, the results of the present study show the need for further investigation into the anti-dyslipidemic activity of isovaleric acid in animals and humans.

Conclusion
Isovaleric acid is as effective as atorvastatin in preventing diet-induced dyslipidemia in rats. Further research on its anti-dyslipidemic potential is warranted in animals and humans.

Conflict of interest
The authors declare no conflict of interest.

References
7. Eadie MJ. Could valerian have been the first anticonvulsant?.Epilepsia. 2004 Nov;45(11):1338-43.


