ABSTRACT

Background: Hyperlipidemia is an important public health problem with increasing incidence and prevalence worldwide. D5 Chooranam is a synergetic Siddha polyherbal formulation designed by Central Council for Research in Siddha which is under patent process. Materials and Methods: In this study assessment of antihyperlipidemic activity of D5 Chooranam was carried out using high fat diet-induced hyperlipidemic model. After 8 weeks of induction hyperlipidemia, the male Wistar rats were treated orally with 250 mg/kg, 500 mg/kg and 750 mg/kg of D5 Chooranam for 6 weeks. Results: The results demonstrated significant hypolipidemic activity by lowering total cholesterol, Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), and triglycerides and by increasing HDL levels compared to hyperlipidemic rats. Atorvastatin (10 mg/kg) was used as a positive control. The reduced AST and ALT levels showed its protective role against hepatic damage. Histopathological findings in rat liver supported the protective role of D5 Chooranam in HFD-induced fatty liver. No significant changes in the renal and cardiac markers provide evidence that D5 Chooranam may not possess serious side effects. Conclusion: Therefore, the study suggests that D5 Chooranam could be effective in treating hyperlipidemia.

Keywords: Cholesterol, High fat diet, Hyperlipidemia, Low density lipoprotein, HDL, Siddha formulation.

INTRODUCTION

Hyperlipidemia has recently emerged as a global concern[1] and is regarded as one of the world’s five dominant causes of death.[2] It is defined as a lipid metabolism disorder characterized by an increase in one or more of the plasma lipids, including Triglycerides (TG), cholesterol, cholesterol esters, phospholipids and or plasma lipoproteins, including Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL) along with diminished HDL levels. Its prevalence is influenced by the genetic and even lifestyle factors such as a high calorie diet and a high intake of cholesterol and saturated fats.[3] Furthermore, it is a vital endangerment leading to fatty liver, cardiovascular disease and atherosclerosis.[4]

According to the global health observatory of WHO, high cholesterol levels are found to be the cause of one-third of ischemic cardiac diseases. The rise in cholesterol has resulted in 2.6 million deaths and 29.7 million disability-adjusted life years.[5] Despite the availability of a wide range of allopathic hypolipidemic like statins and fibrates, they have not gained popularity due to their significant side effects such as rhabdomyolysis, hyperuricemia, hepatotoxicity.[6] Considering the wide magnitude of lipid disorders and the absence of promising remedies with safety in conventional systems, the suffering population is turning towards other alternatives for safe and effective remedies.

Medicinal plants are comprised of plenty of chemical compounds which are the most important source of therapeutic representatives to cure human diseases.[7] D5 Chooranam is a synergetic polyherbal formulation designed by Central Council for Research in Siddha which is under patent process (Application No: IN2578CH2015). In the present study, a high-fat diet induced hyperlipidemic model was used to evaluate the effect of D5 Chooranam as an anti-hyperlipidemic agent.

MATERIALS AND METHODS

Test and standard drug

The test compound, D5 Chooranam was collected from the Pharmacy of Siddha Central Research Institute (CCRS), Arumbakkam, Chennai 600106. The standard drug Atorvastatin was procured from Aurobindo Laboratories, Visakhapatnam.
Preparation of test drug doses

The test drug doses were prepared by triturating a weighed quantity of Chooranam in 25 mL of 0.1% sodium carboxy methyl cellulose (SCMC) to obtain a concentration of 50 mg/mL (250 mg/kg); 75 mg/mL (500 mg/kg) and 100 mg/mL (750 mg/kg). Atorvastatin was prepared by triturating 25 mg of drug in 25 mL of 0.1% SCMC to attain a concentration of 1 mg/mL.

Selection of animal

Healthy male albino rats of Wistar strain weighing about 80 to 120 g were used for the study. The rats have been procured from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai. Animals were housed in polypropylene cages with metallic lid using sterilized paddy husk as bedding material. Relative humidity and temperature were maintained at 55 to 65% and 18 to 25ºC respectively. This study was performed as per the recommendations of the CCSEA guidelines for Laboratory Animal Facility after approval of Institutional Animal Ethics Committee (IAEC) of Siddha Central Research Institute, Central Council for Research in Siddha (Ministry of Ayush), Arumbakkam, Chennai with approval No.167/PHARMA/SCRI/2017.

Induction of Hyperlipidemia

The high-fat diet, with composition as per Table 1, used for this study was procured from the National Institute of Nutrition, Hyderabad, Telangana.[8]

Grouping and Randomization

In this study 36 male albino rats of Wistar strain were used for a period of 16 weeks. The animals were divided to two groups initially i.e., Group A containing 6 animals served as normal group fed with normal diet and Group B containing 30 animals fed with high fat diet for 8 weeks. After 8 weeks, lipid profile was measured to confirm induction of hyperlipidemia, then the 30 animals in Group B were further grouped into 5 groups (II, III, IV, V and VI) containing 6animals each group and Group A continued as Group I (normal group). After 6 weeks of treatment (i.e., after completion of 14th week) half of the animals in each group were euthanized and remaining half animals were kept to know the post treatment effect of D5 Chooranam on organs (liver, heart, pancreas, kidney and aorta) for a period of 2 weeks (15th and 16th week). The animals in the post recovery period received only normal diet and water.

Experimental Design

The animals were divided into 6 groups having 6 animals in each group as follows:

Group I: Normal control.
Group II: High fat diet group.
Group III: Standard group (10 mg/kg-Atorvastatin) (p.o)+HFD.
Group IV: Test Group (Low dose: 250 mg/kg D5 Chooranam) (p.o)+HFD.
Group V: Test Group (Medium dose: 500 mg/kg D5 Chooranam) (p.o)+HFD.
Group VI: Test Group (High dose: 750 mg/kg D5 Chooranam) (p.o)+HFD.

Bodyweight, Feed and water consumption

A body weight of each rat was measured weekly. Water and feed consumption was recorded as weekly throughout the study.

Estimation of Biochemical Parameters

The serum separated from the blood samples collected by puncturing retro-orbital venous plexus was used for estimation of biochemical parameters including the lipid profile, liver markers, renal markers and cardiac markers.[9] Serum triglycerides, total cholesterol, LDL, HDL were calculated using spectrophotometry. VLDL was calculated using Friedwald’s formula.[10] The quantification of serum liver injury markers such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), total protein, albumin, total bilirubin, renal injury markers such as creatinine,[11] urea,[12] cardiac injury markers such as creatine kinase-MB and lactate dehydrogenase was estimated spectrophotometrically.[13]

<table>
<thead>
<tr>
<th>Table 1: Composition of High Fat Diet.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Component</strong></td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Malto-dextrin</td>
</tr>
<tr>
<td>Cellulose</td>
</tr>
<tr>
<td>Soybean oil</td>
</tr>
<tr>
<td>Lard</td>
</tr>
<tr>
<td>L-Cysteine</td>
</tr>
<tr>
<td>*Mineral mix</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
</tr>
<tr>
<td>Calcium carbonate</td>
</tr>
<tr>
<td>Potassium citrate monohydrate</td>
</tr>
<tr>
<td>#Vitamin mixture</td>
</tr>
<tr>
<td>Choline bitartrate</td>
</tr>
</tbody>
</table>

*Mineral mix contains sucrose (1.79 g), potassium citrate (3.3 g), calcium phosphate (2.6 g), calcium carbonate (1.1 g), sodium chloride (0.58 g), magnesium sulphate (0.51 g), magnesium oxide (0.08 g), ferric citrate (0.042 g), magnesium carbonate (0.025 g), zinc carbonate (0.01 g), chromium potassium sulphate (0.004 g), copper carbonate (0.001 g) and ammonium molybdate tetra-hydrate (0.001 g).
#Vitamin mix contains sucrose (7.8 g), vitamin E (1.0 g), niacin (0.3 g), biotin (0.2 g), pantothenic acid (0.17 g), vitamin D3 (0.1 g), vitamin B12 (0.1 g), vitamin A (0.08 g), pyridoxine HCl (0.07 g), riboflavin (0.06 g), thiamine (0.06 g), folic acid (0.02 g) and menadione sodium bisulfate (0.01 g).
Blood pressure
The blood pressure was measured by using the non-invasive blood pressure apparatus following the tail cuff method.\textsuperscript{[14]}

Histopathology
The animals were sacrificed after 14 weeks i.e., 6 weeks of treatment and the organs were isolated and observed them macroscopically for abnormalities viz color, cell damage etc. The tissues and organs preserved in 10% neutral formalin were sectioned with microtome, and stained with hematoxylin and eosin to study histopathological changes.\textsuperscript{[15]}

Statistical analysis
All the data was expressed as mean±SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey’s post parametric test by GraphPad prism software version5. The significance level was set at $p$ value $<0.05$ for all tests.\textsuperscript{[16]}

RESULTS
Effect of D5 Chooranam on body weight, food and water intake
The graph shows the effect of D5 Chooranam on body weight of experimental and normal groups of animals. The increase in body weight of Group II over Group I was statistically significant ($p<0.05$). However, D5 Chooranam treated animals tend not to significantly differ from Group II even at the 6th week of study. There were no statistical differences between the groups regarding feed intake. The water intake was significantly higher ($p<0.05$) in the 6th week of treatment period when D5 Chooranam treated groups were compared to Group II.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Effect of D5 Chooranam on (A): Body weight, (B): Food intake and (C): Water intake of the HFD fed animals after 6 weeks of treatment. Values represent Mean±SEM of animals in each group, where $n=6$. *denotes $p<0.05$ when compared to group I, # denotes $p<0.05$ when compared to group II.}
\end{figure}
Effect on Lipid profile
The levels of serum total cholesterol, triglyceride, LDL, VLDL of high fat diet treated animals increased significantly ($p<0.05$) with a concomitant decrease in HDL when compared with the control rats. The changes in the lipid profile were attenuated in high fat diet treated animals after administration of Atorvastatin (10 mg/kg), 200, 500, 750 mg/kg of D5 Chooranam as represented in Table 2.

Effect of D5 Chooranam on liver function test
As shown in Figure 2, D5 Chooranam treated groups showed a significant ($p<0.05$) decrease in SGOT, SGPT levels compared to Group II. Animals treated with 500 mg/kg of D5 Chooranam showed a tremendous fall in alkaline phosphatase levels when compared to HFD fed animals. Also, no significant difference in total protein level, serum albumin, and total bilirubin levels in Group II compared to Group I was observed. The same trend was observed when D5 Chooranam treated groups were compared to Group II.

Effect of D5 Chooranam on renal function test
No statistical differences were observed in serum urea levels between the normal, HFD fed and treatment groups. The animals treated with 750 mg/kg of D5 Chooranam displayed a significant ($p<0.05$) decrease in serum creatinine levels when compared to Group II as shown in Figure 3.

Effect of D5 Chooranam on cardiac markers
As shown in Figure 4, a significant ($p<0.05$) decline in Ck-MB (Creatine kinase-MB) in D5 Chooranam treated groups compared to Group II was detected. A significant ($p<0.05$) increase in lactate dehydrogenase levels was noticed in Group II compared to Group I. Whereas the same levels had shown a significant ($p<0.05$) decline in D5 Chooranam treated Group V as comparable to Group II.

Effect of D5 Chooranam on blood pressure
As shown in Table 3, a significant ($p<0.05$) decrease was noticed in systolic and diastolic blood pressure when Group II compared to Group I whereas the levels were significantly ($p<0.05$) improved respectively in D5 Chooranam treated groups compared to Group II.

Effect on relative organ weight
The relative organ weights are presented in Table 4. No significant difference in the relative weight of liver, heart was noted between the groups whereas D5 Chooranam treated groups significantly ($p<0.05$) increased the relative weight of kidney, decreased the relative weight of pancreas, aorta significantly ($p<0.05$) when compared to Group II.

Histopathology
The findings of the histopathological examination of liver, heart, kidney, pancreas and aorta of animals fed with high fat diet, standard drug and the D5 Chooranam (250, 500 and 750 mg/kg) at the end of the treatment period are shown in Figures 5-8. The histopathology of liver of Group I showed normal characteristic features radiating from central vein. The Group II showed congestion, multifocal moderate vesicular (micro to macro) fatty degeneration of hepatocytes. The standard and test treated groups

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**Table 2:** Effect of D5 Chooranam on Lipid profile of the HFD fed animals after six weeks of treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>HDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>63.2±4.0</td>
<td>67.8±8.2</td>
<td>23.4±1.5</td>
<td>23.4±1.5</td>
<td>26.2±1.9</td>
</tr>
<tr>
<td>Group II</td>
<td>82.2±4.1*</td>
<td>99.6±3.2*</td>
<td>37±1.2</td>
<td>37±1.2*</td>
<td>27.8±8.3*</td>
</tr>
<tr>
<td>Group III</td>
<td>77±8.9</td>
<td>75.2±1.2*</td>
<td>22.3±3.7*</td>
<td>22.3±3.7*</td>
<td>39.6±5.3*</td>
</tr>
<tr>
<td>Group IV</td>
<td>76±5.6*</td>
<td>105±3.1</td>
<td>24.8±2.8*</td>
<td>24.8±2.8*</td>
<td>34±6.1*</td>
</tr>
<tr>
<td>Group V</td>
<td>76±3.8*</td>
<td>73.2±3.2*</td>
<td>26.2±2.2*</td>
<td>26.2±2.2*</td>
<td>33.5±11.5*</td>
</tr>
<tr>
<td>Group VI</td>
<td>79.6±5.3*</td>
<td>146.8±6.1*</td>
<td>25.2±3.4*</td>
<td>25.2±3.4*</td>
<td>32.3±3.9*</td>
</tr>
</tbody>
</table>

Values represent Mean±SEM of animals in each group, where $n=6$.* denotes $p<0.05$ when compared to Group I, # denotes $p<0.05$ when compared to Group II.
Reddy, et al.: Hyperlipidemic Activity of D5 Chooranam

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Table 4: Effect of D5 Chooranam on relative organ weights of the HFD fed animals after 6 weeks of treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
<th>Pancreas</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>44.45±0.29</td>
<td>4.36±0.21</td>
<td>8.91±0.10</td>
<td>3.77±0.1</td>
<td>0.84±0.09</td>
</tr>
<tr>
<td>Group II</td>
<td>39.87±0.92</td>
<td>3.66±0.08</td>
<td>8.37±0.02</td>
<td>3.29±0.11</td>
<td>0.76±0.04</td>
</tr>
<tr>
<td>Group III</td>
<td>47.27±3.00</td>
<td>3.26±0.06</td>
<td>9.01±0.50</td>
<td>2.85±0.39</td>
<td>0.54±0.06</td>
</tr>
<tr>
<td>Group IV</td>
<td>41.45±0.94</td>
<td>3.90±0.12</td>
<td>9.23±0.18</td>
<td>2.99±0.35</td>
<td>0.54±0.07</td>
</tr>
<tr>
<td>Group V</td>
<td>40.50±3.05</td>
<td>4.34±0.02</td>
<td>8.50±0.20</td>
<td>2.62±0.24</td>
<td>0.86±0.02</td>
</tr>
<tr>
<td>Group VI</td>
<td>40.50±2.64</td>
<td>4.15±0.02</td>
<td>9.32±0.75</td>
<td>2.90±0.36</td>
<td>0.88±0.20</td>
</tr>
</tbody>
</table>

Values represent Mean±SEM of animals in each group, where n=6.*denotes p<0.05 when compared to Group II.

**Table 4:** Effect of D5 Chooranam on relative organ weights of the HFD fed animals after 6 weeks of treatment.

**Figure 8:** Effect of D5 Chooranam on aorta histopathology of the HFD fed animals after six weeks of treatment. (A) Normal, (B) Disease Control, (C) Atorvastatin 10 mg/kg, (D) D5 Chooranam 250 mg/kg, (E) D5 Chooranam 500 mg/kg and (F) D5 Chooranam 750 mg/kg. Values represent Mean±SEM of animals in each group, where n=6.

hyperlipidemic activity of D5 Chooranam

Reddy, et al.: Hyperlipidemic Activity of D5 Chooranam

The standard and test treated groups had showed that the tubular epithelial cells damage reduced from moderate to mild when compared with Group II. The pancreas histopathology of Group I were found to be normal, while the pancreas of Group II was showed moderate generation of pancreatic acinar cells resulting in pancreatitis. The standard and test treated groups had showed that the degeneration of pancreatic acinar cells reduced from moderate to mild when compared Group II; it is evident that the test drug has reduced pancreatitis. Histopathology of heart of normal animals hadn't shown any cellular degeneration and vacuole formation. In the hyperlipidemic control group animals the heart histopathology shows formation of vacuoles in myocytes and also severe degeneration of myocytes. When compared with the hyperlipidemic control group animals the test groups and standard were effective in reducing the myocardial degeneration and vacuole formation. The myocardial degeneration and vacuoles formation results in the cardiac disorders. The aorta of Group II had shown multifold vacuoles in tunica media and the Group I had not shown any abnormalities. The test dose treated groups and atorvastatin treated groups were shown normal characteristic features without any abnormalities when compared with Group II.

**DISCUSSION**

Hyperlipidemia is one of the major causes of mortality and has been documented as one of the most important risk factors for several disorders like atherosclerosis, coronary artery disease, hypertension and type 2 diabetes mellitus. This study assessed the anti-hyperlipidemic effect of D5 Chooranam in high fat diet induced model. HFD-fed hyperlipidemic rat model has been reported previously as an ideal in vivo model for testing antihyperlipidemic drugs. The male albino rats of Wistar
strain were selected as the test system instead of females as their hormonal regulation is protective for the development of hyperlipidemia.\textsuperscript{[21]} HFD induced hyperlipidemia causes rise in the levels of total cholesterol, TG, LDL, VLDL and reduced HDL levels. The elevated lipid profile is indicative of successful induction of hyperlipidemia. In the present study, D5 Chooranam at 750 mg/kg substantially decreased the total cholesterol, LDL, and VLDL levels, and increased the HDL level in hyperlipidemic animals as shown in Table 3. Enzymes like ALT, AST and ALP are considered as marker enzymes to elucidate the integrity and function of liver.\textsuperscript{[22]} It is evident from previous studies that hypercholesterolemia might induce liver damage.\textsuperscript{[23]} D5 Chooranam at a dose of 500 mg/kg substantially decreased the levels of SGOT and SGPT when compared to the model group indicates that it can bypass the complication of statins and improves the fatty liver as shown.\textsuperscript{[24]} The increase in ALP activity following feeding a HFD suggests disruption of lipid-bilayer of the membrane structures of the affected organs. It was noted that D5 Chooranam at a dose of 500 mg/kg normalized the ALP activity than atorvastatin in hyperlipidemic rats. Collectively, results from this present study suggest that D5 Chooranam treatment restored the liver function in hyperlipidemic rats. Serum urea and creatinine concentrations are the primary prognostics in kidney damage.\textsuperscript{[25]} Hence, the role of D5 Chooranam in combating the renal aberrations accompanying HFD induced hyperlipidemia has been investigated. Animals fed with HFD shown increase in creatinine levels when compared to control animals and is associated with nephritic changes occurring in the renal tissue. D5 Chooranam at 500 mg/kg significantly decreases the serum creatinine levels in hyperlipidemic rats. HFD disrupts the normal functioning of heart by causing damage to cardiac myocytes leading to cardiac injury.\textsuperscript{[26]} D5 Chooranam at 750 mg/kg significantly decreased the Ck-MB levels in hyperlipidemic rats. This is indicative that the D5 Chooranam has effective in normalizing the cardiac markers and offers cardiac protection. Parallel to the alterations observed in biochemical parameters, light microscopical examination revealed that the treatment of D5 Chooranam played a remarkable role in lowering the condition of fatty liver showing mild damage of hepatocytes when compared to HFD fed animals.

This study has showed that a combination of various herbs of D5 Chooranam resulted in significant improvement in lipid levels, cardiac and liver markers without any significant serious adverse effects. Human trials with relevant clinical end points are required to establish the efficacy of D5 Chooranam.

CONCLUSION

D5 Chooranam, a Siddha poly herbal formulation found to be effective in the management of hyperlipidemia at three dose levels 250, 500, 750 mg/kg as it was able to decrease the TG, LDL, VLDL, and increase the HDL levels. D5 Chooranam at 3 dose levels didn’t show any adverse effects in hyperlipidemic rats. Hence, the chemical constituents of the test drug might help in preventing hyperlipidemic complications and may serve as an alternative in management of hyperlipidemia. Further study on D5 Chooranam is needed to substantiate the mechanism involved in treating hyperlipidemia and to know the effect in cardiovascular disorders, obesity and metabolic syndrome.

ACKNOWLEDGEMENT

We are grateful for the financial support from Central Council for Research in Siddha (CCRS), Ministry of Ayush for performing this study. We are thankful to all the staffs of Department of Pharmacology for providing their support for carrying out this research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this paper.

ABBREVIATIONS

LDL: Low density lipoprotein; VLDL: Very low-density lipoprotein; TG: Triglycerides; HFD: High Fat Diet; CCRS: Central Council for Research in Siddha; SCMC: Sodium carboxy methyl cellulose; TANUVAS: Tamil Nadu Veterinary and Animal Sciences University; IAEC: Institutional Animal Ethics Committee; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase.

SUMMARY

D5 Chooranam, a Siddha poly herbal formulation found to be effective in the management of hyperlipidemia at three dose levels in hyperlipidemic rats. Further study on D5 Chooranam is needed to substantiate the mechanism involved in treating hyperlipidemia and to know the effect in cardiovascular disorders, obesity and metabolic syndrome.

REFERENCES