ANTI-HYPERLIPIDEMIC ACTIVITY OF AQUEOUS EXTRACT OF *TERMINALIA CHEBULA* & GAUMUTRA IN HIGH CHOLESTEROL DIET FED RATS

Dipa A. Israni¹*, Kirti V. Patel², Tejal R. Gandhi²

¹Dept. of Pharmacology, Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa 383315, India.

²Dept. of Pharmacology, Anand Pharmacy College, Anand 388001, India.

ABSTRACT

*Terminalia chebula* RETZ. (Combretaceae), a native plant in India and Southeast Asia commonly known as Haritaki has been reported to exhibit a variety of biological activities. In the present study, aqueous extract of *Terminalia chebula* and its combination with Gaumutra were investigated for anti-hyperlipidemic activity in Sprague-dawley rats. Hyperlipidemia was induced by giving high cholesterol diet (2% cholesterol, 1% sodium cholate and 2% coconut oil) for thirty days in standard rat chow diet. Rats on high cholesterol diet showed significant increase (p<0.05) in serum and tissue cholesterol, LD L-C, V L D L-C, triglyceride, atherogenic index and decrease HD L-C levels. Treatment with *Terminalia chebula* (300mg/kg, p.o) and its combination with Gaumutra (30mg/kg, p.o) showed significant decrease (p<0.05) in serum and tissue serum and tissue cholesterol, LD L-C, V L D L-C, triglyceride, atherogenic index and increase HD L-C levels. Histological study showed that *Terminalia chebula* caused decrease in aortic plaque and fatty liver formation but its combination with Gaumutra showed no significant effect in aorta and liver as compared to high cholesterol diet fed rats. Thus *Terminalia chebula* and its combination with Gaumutra both are effective as an anti-hyperlipidemic agent.

Keywords: *Terminalia chebula*; Gaumutra; high cholesterol diet

INTRODUCTION

The American Heart Association have identified the primary risk factor associated with the atherosclerosis is the elevated levels of cholesterol and triglyceride in the blood. Therefore the therapist considers the treatment of hyperlipidemia to be one of the
major approaches towards decelerating the atherogenic process \[1\]. Atherosclerosis, referred to as a “silent killer”, is one of the leading causes of death in the developing countries like India \[2\]. In the general population, the cardiovascular disease risk from increased LDL cholesterol is supported by observations that cholesterol-lowering therapy greatly diminishes the clinical manifestations of atherosclerosis, particularly since the advent of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (i.e., statins) that profoundly lower LDL cholesterol \[3\]. In contrast to the situation with LDL cholesterol, the relation between HDL cholesterol and atherosclerosis is an inverse one \[4\].

Allopathic hypolipidemic drugs are available at large in the market but the side-effects and contraindications of these drugs have marred their popularity. Recently herbal hypolipidemics have gained importance to fill the lacunae created by the allopathic drugs \[5\].

*Terminalia chebula* RETZ. (Combretaceae), a native plant in India and Southeast Asia, is extensively cultivated in Taiwan. Its dried ripe fruit, also called as medicinal terminalia fruit, has traditionally been used to treat various ailments in Asia \[6\]. *Terminalia chebula* (Kadukkai) is one of the traditional Ayurvedic medicines that have found to possess various qualities on curing different kinds of diseases. *T. chebula* has been reported to exhibit a variety of biological activity, including anticancer, antidiabetic, antimutagenic, antibacterial, antifungal, and antiviral activities, etc \[7\]. In ayurveda it is mention that *T. chebula* is an effective anti-hyperlipidemic agent \[8\] & as per ayurvedic doctors addition of Gaumutra may enhance the efficacy of *Terminalia chebula*. Fruit of *Terminalia chebula* contains Tannins, anthraquinones, chebulinic acids, chebulic acid, ellagic acid and gallic acid also possesses corilaegin, β-D-glucogallin, glucose and sorbitol\[9\]. In light of above objective, current investigation was carried to study effect of aqueous extract of *Terminalia chebula* fruits and its combination with gaumutra in high cholesterol diet induced hyperlipidemia in rats.

**MATERIAL & METHODS**

**Plant material**

Dried ripe fruits of *Terminalia chebula* were obtained from a commercial supplier and it was identified and authenticated by Mr. M. S. Jangid, Botany
Department, Modasa, India. A voucher specimen was retained in our laboratory for further reference. Aqueous extract of *Terminalia chebula* was prepared by grinding 600 g of fruits into small pieces, and then extracted with 1 liter of warm water for 24 h. The same procedure was repeated another two times. The extracts were then combined, concentrated under reduced pressure, and finally dried. The final yield of the water extract was 137.0 g. and Gaumutra extract was obtained from Aarkay food products, Ahmedabad. TLC was run for authentication of extract and Rf value were comparable with that of standard.

**Animals**

Sprague Dawley rats weighing 200-250 gm of either sex were used. Rats were maintained on a standard diet and water *ad libitum*. All animals were housed at ambient temperature (21±1°C) and relative humidity (55±5%) with fixed 12h/12h light/dark cycle. Animals had free access to standard pellet diet and water given *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

**Treatment protocols**

Dose of *Terminalia chebula* was selected by using the references of various articles on *T. chebula*, further 7 days Anti-hyperlipidemic study was done using four different doses 100mg/kg, 300mg/kg, 500mg/kg and 700mg/kg of *T. chebula*. Among this dose 300mg/kg showed good anti-hyperlipidemic activity. So 300mg/kg of *T. chebula* was selected. Similarly as per ayurvedic physician *T. chebula* combined with 10% of guamutra may show good activity. Thus, 7 days study was done using four combinations, i.e., *T. chebula* (90mg/kg) + Gaumutra(10mg/kg); *T. chebula* (270mg/kg) + Gaumutra(30mg/kg); *T. chebula* (450mg/kg) + Gaumutra(50mg/kg) and *T. chebula* (6300mg/kg) + Gaumutra(70mg/kg). Among this combinations, *T. chebula* (270mg/kg) + Gaumutra(30mg/kg) showed good anti-hyperlipidemic activity.

Hyperlipidemia in rats was induced by administration of high cholesterol diet (2% cholesterol, 1% sodium cholate and 2% coconut oil) for thirty days in standard rat chow diet. The rats were divided into five groups; each group containing six rats. Group 1
served as normal control and received saline solution. Group 2 High Cholesterol diet – Cholesterol control (HCD) for 30 days. Group 3 received High Cholesterol diet (HCD) + *Terminalia chebula* (300 mg/kg, p.o. in Distilled water) for 30 days. Group 4 received High Cholesterol diet (HCD) + *Terminalia chebula* (270 mg/kg, p.o. in Distilled water) & Gaumutra (30 mg/kg, p.o. in distilled water) for 30 days. Group 5 received High Cholesterol diet (HCD) + Atorvastatin (15mg/kg, p.o. in CMC) – Standard control for 30 days.

Blood were collected initially before the administration of the diet i.e. on 0 day and After 30 days. Blood samples were collected from the tail vein after 8 h fast and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimation of lipid profile. Serum lipid profile was then estimated (Auto-span diagnostic kits pvt. Ltd., India) \(^{10, 11}\). Liver, Heart and Thoracic aorta was dissected out from rats of each group. Tissue were rinsed with distilled water and homogenate in chloroform:methanol (2:1v/v) mixture. This homogenate were further processed for estimation of lipid profile \(^{12}\). Food intake and Weight gain in rats of each group were observed for 30 days. Samples of aorta and liver were collected from the each group of animals for histopathology.

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Result were analyzed statistically using Student’s unpaired and paired t-test wherever applicable. Values of p< 0.05 were considered significant.

RESULTS

Effect on Food intake and weight gain

The food intake and weight was increased in high cholesterol fed diet rats as compared to normal control. Treatment with aqueous extract of *T. chebula* and its combination with Gaumutra showed no change in food intake but there was significant decrease in weight gain as compared to high cholesterol fed diet rats (Table-1).
TABLE-1 EFFECTS OF AQUEOUS EXTRACT OF T. CHEBULA AND ITS COMBINATION WITH GAUMUTRA ON CHANGE IN BODY WEIGHT

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CHANGE IN THE BODY WEIGHT (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>11.67 ± 7.032</td>
</tr>
<tr>
<td>Cholesterol control (HFD)</td>
<td>51.67± 14 *</td>
</tr>
<tr>
<td>HFD + T. chebula treated (300mg/kg)</td>
<td>-18.33± 4.773 $^\circ$</td>
</tr>
<tr>
<td>HFD + [T. chebula (270mg/kg) + Gaumutra (30mg/kg)]</td>
<td>-1.667± 6.54 $^\circ$</td>
</tr>
<tr>
<td>HFD + Statin treated (15mg/kg)</td>
<td>-31.67± 4.773 $^\circ$</td>
</tr>
</tbody>
</table>

All the values are expressed in Mean ± SEM of 6 observations.

(student’s unpaired t-test)

*Significant different as compared to normal control P<0.05.

$ Significant different as Compared to cholesterol control P<0.05

Effect on Serum lipid profile

High Cholesterol diet (HCD) fed rats produced significant increase (p<0.05) in serum cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index but significant decrease (p<0.05) in HDL-C level as compared to 0 day and normal control rats (Table-2). Treatment with aqueous extract of T.chebula & its combination with gaumutra showed significant reduction (p<0.05) in serum cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index but significant increase (p<0.05) in HDL-C as compared to high cholesterol diet fed rats (Table-2).

Effect on Tissue lipid profile

High Cholesterol diet (HCD) fed rats produced significant increase (p<0.05) in tissue cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index but decrease in HDL-C level as compared to 0 day and normal control rats (Table-3). Treatment with aqueous extract of T.chebula & its combination with Gaumutra showed significant reduction (p<0.05) in tissue cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic index but increase in HDL-C as compared to high cholesterol diet fed rats (Table-3).
### TABLE-2 EFFECTS OF AQUEOUS EXTRACT OF T. CHEBULA AND ITS COMBINATION WITH GAUMUTRA ON SERUM LIPID PROFILE

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>VLDL cholesterol</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>65.88 ± 6.945</td>
<td>77.6 ± 1.947</td>
<td>32.79 ± 5.818</td>
<td>26.6 ± 7.5</td>
<td>15.49 ± 0.3878</td>
<td>16.26 ± 0.8492</td>
</tr>
<tr>
<td>After 30 day</td>
<td>79.26 ± 4.267</td>
<td>78.87 ± 6.191</td>
<td>39.28 ± 3.735</td>
<td>24.24 ± 3.41</td>
<td>15.77 ± 1.239</td>
<td>16.43 ± 1.227</td>
</tr>
<tr>
<td>Cholesterol control (HFD)</td>
<td>64.05 ± 3.093</td>
<td>93.66 ± 3.39</td>
<td>37.77 ± 3.016</td>
<td>14.29 ± 3.184</td>
<td>20.24 ± 1.787</td>
<td>21.75 ± 1.827</td>
</tr>
<tr>
<td>After 30 day</td>
<td>375.3 ± 18.49</td>
<td>164.9 ± 19.76</td>
<td>24.91 ± 2.027</td>
<td>319.8 ± 16.61</td>
<td>20.24 ± 1.787</td>
<td>36.92 ± 1.208</td>
</tr>
<tr>
<td>HFD + T. chebula treated</td>
<td>46.92 ± 2.941</td>
<td>75.91 ± 4.367</td>
<td>22.74 ± 1.061</td>
<td>9.935 ± 3.119</td>
<td>14.47 ± 0.6525</td>
<td>16.1 ± 1.116</td>
</tr>
<tr>
<td>After 30 day</td>
<td>225.5 ± 14.64</td>
<td>101.2 ± 8.933</td>
<td>54.62 ± 5.055</td>
<td>166 ± 18.74</td>
<td>20.24 ± 1.787</td>
<td>27.29 ± 3.004</td>
</tr>
<tr>
<td>HFD + T. chebula + Gaumutra treated</td>
<td>57.18 ± 8.181</td>
<td>73.09 ± 8.339</td>
<td>26.27 ± 2.979</td>
<td>13.01 ± 5.891</td>
<td>14.62 ± 1.668</td>
<td>17.72 ± 0.939</td>
</tr>
<tr>
<td>After 30 day</td>
<td>252 ± 40.39</td>
<td>107.9 ± 4.991</td>
<td>49.85 ± 2.841</td>
<td>185.6 ± 41.41</td>
<td>21.58 ± 0.9985</td>
<td>28 ± 1.961</td>
</tr>
<tr>
<td>HFD + Statin treated</td>
<td>59.58 ± 3.948</td>
<td>64.93 ± 7.972</td>
<td>29.95 ± 1.914</td>
<td>17.42 ± 3.648</td>
<td>12.98 ± 1.594</td>
<td>14.54 ± 1.834</td>
</tr>
<tr>
<td>After 30 day</td>
<td>173.5 ± 5.512</td>
<td>98.41 ± 4.412</td>
<td>62.87 ± 5.143</td>
<td>89.15 ± 5.922</td>
<td>19.68 ± 0.8833</td>
<td>22.35 ± 1.165</td>
</tr>
</tbody>
</table>

All the values are expressed in Mean ± SEM of 6 observations.

(student’s unpaired t-test)

*Significant different as compared to normal control P<0.05

$ Significant different as Compared to cholesterol control P<0.05

**Histological results of aorta**

In the histopathological study high cholesterol diet fed rats exhibit atheromatous plaque as compared to normal control. Treatment with aqueous extract of T. chebula shows decrease in plaque size as compared to cholesterol control. Combination of T. chebula with Gaumutra shows fibrofatty plaque in aorta (Fig. 1).
TABLE-3 EFFECTS OF AQUEOUS EXTRACT OF *T. CHEBULA* AND ITS COMBINATION WITH GAUMUTRA ON TISSUE LIPID PROFILE

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Cholesterol</th>
<th>Cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>VLDL cholesterol</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Liver</td>
<td>32.46 ± 3.253</td>
<td>87.79 ± 3.457</td>
<td>17.53 ± 1.669</td>
<td>1.184 ± 0.555</td>
<td>17.55 ± 0.6932</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>41.94 ± 1.978</td>
<td>60.21 ± 3.095</td>
<td>14.02 ± 1.552</td>
<td>16.8 ± 3.883</td>
<td>12.04 ± 0.6191</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>46.4 ± 1.96</td>
<td>46.94 ± 5.114</td>
<td>8.818 ± 1.463</td>
<td>5.748 ± 2.08</td>
<td>9.383 ± 1.023</td>
</tr>
<tr>
<td>Cholesterol control (HFD)</td>
<td>Liver</td>
<td>104.6 ± 8.163 *</td>
<td>139.6 ± 6.23 *</td>
<td>8.598 ± 2.258 *</td>
<td>59.08 ± 0.2094 *</td>
<td>30.73 ± 3.112 *</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>102.3 ± 2.397 *</td>
<td>139.6 ± 6.23 *</td>
<td>7.144 ± 0.4648 *</td>
<td>68.85 ± 4.573 *</td>
<td>27.91 ± 1.246 *</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>115.7 ± 6.926 *</td>
<td>138.7 ± 4.046 *</td>
<td>32.33 ± 2.375 *</td>
<td>65.95 ± 3.33 *</td>
<td>27.74 ± 0.8083 *</td>
</tr>
<tr>
<td>HFD + <em>T. chebula</em></td>
<td>Liver</td>
<td>53.81 ± 2.834 $</td>
<td>84.5 ± 2.439 $</td>
<td>16.86 ± 1.067 $</td>
<td>20.04 ± 3.613 $</td>
<td>17.6 ± 0.7834 $</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>41.43 ± 2.163 $</td>
<td>63.23 ± 3.621 $</td>
<td>19.83 ± 1.518 $</td>
<td>11.36 ± 2.502 $</td>
<td>12.64 ± 0.7241 $</td>
</tr>
<tr>
<td>HFD + <em>T. chebula</em> + Gaumutra</td>
<td>Liver</td>
<td>55.33 ± 2.857 $</td>
<td>92.95 ± 4.304 $</td>
<td>9.017 ± 1.264 $</td>
<td>27.69 ± 2.066 $</td>
<td>19.22 ± 0.8774 $</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>62.22 ± 4.021 $</td>
<td>78.87 ± 4.265 $</td>
<td>17.72 ± 0.6412 $</td>
<td>29.43 ± 2.507 $</td>
<td>14.67 ± 0.8963 $</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>69.93 ± 4.352 $</td>
<td>66.66 ± 3.459 $</td>
<td>20.66 ± 2.013 $</td>
<td>32.69 ± 4.07 $</td>
<td>14.57 ± 1.335 $</td>
</tr>
<tr>
<td>HFD + Statin treated</td>
<td>Liver</td>
<td>49.67 ± 3.977 $</td>
<td>61.44 ± 4.372 $</td>
<td>18.54 ± 1.563 $</td>
<td>15.39 ± 2.23 $</td>
<td>10.75 ± 0.6313 $</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>35.29 ± 1.887 $</td>
<td>53.75 ± 3.157 $</td>
<td>33.75 ± 6.05 $</td>
<td>7.763 ± 3.117 $</td>
<td>10.2 ± 0.4745 $</td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>36.27 ± 1.718 $</td>
<td>43.19 ± 2.484 $</td>
<td>29.03 ± 4.867 $</td>
<td>3.4 ± 6.52 $</td>
<td>8.638 ± 0.4968 $</td>
</tr>
</tbody>
</table>

All the values are expressed in Mean ± SEM of 6 observations.

(student’s unpaired t-test)

*Significant different as compared to normal control P<0.05

$ Significant different as Compared to cholesterol control P<0.05
In the histopathological study high cholesterol diet fed rats shows fatty cytoplasmic vaculated cells as compared to normal control. Treatment with aqueous extract of *T. chebula* shows less fatty cytoplasmic vacules as compared to high cholesterol diet fed rats. Combination of *T. chebula* with Gaumutra shows focal area of cytoplasmic vacules (Fig. 2).

**Histological results of liver**

**Figure 1**

Histology of Aorta
DISCUSSION

Development of atherosclerotic disease is a complicated process involving accumulation of lipid-containing particles in the walls of coronary arteries & other major arteries within the body. A high-fat diet causes cholesterol levels to increase in susceptible people, which leads to obesity. The weight gain in high cholesterol diet (HCD) group of rats was significantly higher than control rats reflecting the influence of high cholesterol diet [13]. Similarly, in present study there was significant weight gain in cholesterol control (HCD) as compared to normal control groups. Treatment with *T. chebula* and its combination with gaumutra significantly reduced the weight gain. Lowering high cholesterol levels significantly reduce the risk of heart attacks, strokes, and death. Normally hepatocyte initiate synthesis of triglycerides and cholesterol during states of increased free fatty acid flux to the liver (e.g., after the fatty meal or in the situation of increased lipolysis) but due to anti-hyperlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocyte cholesterol concentration by increases the catabolic conversion of cholesterol to bile acids in liver. High chol diet increased serum cholesterol and LDL-C level significantly [10, 14, 15]. A rise in LDL may cause deposition of cholesterol in arteries and aorta and hence it is a direct risk factor for coronary heart disease [16, 17]. Studies show that both LDL and VLDL have a positive role in atherogenesis [16, 18, 19]. In the present study, there was elevation in serum and tissue cholesterol, LDL-C, and VLDL-C level in response to high cholesterol diet as compare to normal control group. Treatment with aqueous extract of
"T. chebula" and its combination with Gaumutra significantly reduced serum and tissue cholesterol, LDL-C, and VLDL-C level.

The decrease in serum triglyceride level is an important finding of this experiment. Recent studies show that triglycerides are independently related with coronary artery disease \[^{[20, 21]}\]. Treatment with aqueous extract of "T.chebula" and its combination with Gaumutra shows significant decreased in triglyceride.

HDL is synthesized mainly in intestine and liver. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. Low level of HDL is associated with high risk of coronary artery disease \[^{[22]}\]. In the present study HDL-C level in both serum and tissue were significantly increased by aqueous extract of "T.chebula" and its combination with gaumutra.

Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the higher is the risk of above organs for oxidative damage \[^{[23]}\]. Treatment with aqueous extract of "T.chebula" and its combination with Gaumutra shows significant decreased in Atherogenic index.

In histopathological study we found treatment of "T. chebula" significantly decreases the plaque size in aorta and significantly decrease fatty cytoplasmic vaculated cells in Liver parenchyma as well as liver cell necrosis is prevented. But combination of "T. chebula" with gaumutra showed no effect in aorta and liver compared to HCD rats.

**CONCLUSION:**

Hence treatment with "T. chebula" and its combination with gaumutra significantly decreases the Cholesterol, Triglyceride, VLDL-C, LDL-C, Atherogenic index and a significantly increase in HDL-C in serum and various tissue homogenate like Aorta, Liver & Heart. These results were further substantiated with the histopathological results. Anti-hyperlipidemic activity of aqueous extract of *Terminalia chebula* may be due to presence of tannins, anthraquinones, chebulinic acids, chebulic acid, ellagic acid and gallic acid and this requires further investigation.

Thus from above results it can be concluded that aqueous extract of *Terminalia chebula* and its combination with gaumutra has significant anti-hyperlipidemic activity.
REFERENCES


16. Pedersen TR. Pro and con: Low density lipoprotein cholesterol lowering is and will be the key to the future of lipid management. Am J Cardiol 2001; 87: 8B-12B.


22. Boden WE and Pearson TA: Raising low levels of high-density lipoprotein cholesterol is an important target of therapy. Am J Cardiol. 2000; 85:645.


For Correspondence:
Ms. Dipa A. Israni
Dept. of Pharmacology, Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa 383315, India.
Email: dip_israni@yahoo.co.in; dipaisrani@gmail.com
Tel. 02774-247160; Fax no. 02774-249482