Objective: The objective of the present study was to investigate the antihyperlipidemic activity of methanolic extract of Bambusa bambos and ethanolic extract of Swertia chirata.

Method: Antihyperlipidemic activity was determined using cholesterol suspension induced hypercholesterolemia in rats. Rats were randomly assigned to five groups: Group I (Vehicle control), Group II (Disease control) 25mg/kg cholesterol in coconut oil, Group III (Standard control) 25mg/kg cholesterol in coconut oil + 10mg/kg Atrorvasatin, Group IV (MEBB) 25mg/kg cholesterol in coconut oil + 300mg/kg methanolic extract of leaves of Bambusa bambos (MEBB), Group V (EESC) 25mg/kg cholesterol in coconut oil + 400mg/kg ethanolic extract of Swertia chirata (EESC). Cholesterol suspension and test drugs were administered for period of 28 days. At the end of experimental period blood was withdrawn from retro-orbital plexus and was used for biochemical estimation of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C). Liver was analysed for antioxidant parameters (lipid peroxidation, reduced glutathione and catalase).

Results: Administration of methanolic extract of Bambusa bambos and ethanolic extract of Swertia chirata significantly (p<0.01) decreased serum TC, TG, LDL-C, VLDL-C levels, atherogenic indices and significantly (p<0.01) increased serum HDL-C level. Lipid peroxidation was significantly (p<0.01) decreased whereas reduced glutathione and catalase levels were significantly (p<0.01) increased in liver.

Conclusion: Methanolic extract of Bambusa bambos and ethanolic extract of Swertia chirata demonstrated effective hypocholesterolemic action together with antioxidant effect.

Keywords: Bambusa bambos, Swertia Chirata, Antihyperlipidemic, Atherogenic indices,
commercial pellet diet (Amrut Laboratory, Mumbai, India) and tap water ad libitum. The animals were acclimatized for one week before the start of the experiment. The experiments were carried out in accordance with the guidelines set by Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

**In vivo antihyperlipidemic activity- cholesterol suspension induced hypercholesterolemia**

The antihyperlipidemic activity was determined by the method of Dhule et al. (1999) [9].

Female Sprague Dawley rats were divided randomly into 5 groups of 6 animals each as follows:

- **Group I:** Vehicle control group in which rats were administered daily with vehicle i.e. 0.5% w/v Sodium CMC solution
- **Group II:** Disease control group in which the rats were administered daily with cholesterol (25 mg/kg/day) in coconut oil.
- **Group III:** Standard control group in which the rats were administered daily with Atorvastatin (10mg/kg/day) along with cholesterol (25 mg/kg/day) in coconut oil.
- **Group IV:** Test group in which the rats were administered daily with methanolic extract of leaves of *Bambusa bambos* (MEBB) (300mg/kg/day) along with cholesterol (25 mg/kg/day) in coconut oil.
- **Group V:** Test group in which rats were administered daily with ethanolic extract of leaves of *Swertia chirata* (EESC) (400mg/kg/day) along with cholesterol (25 mg/kg/day) in coconut oil.

A homogeneous suspension of the extracts and standard drug Atorvastatin was freshly prepared individually using 0.5% Sodium CMC solution. Cholesterol in coconut oil (cholesterol suspension) and the test compounds were administered for period of 28 days. The time interval between administration of cholesterol suspension and test compounds was 5hr. The vehicle control group was treated with vehicle instead of drugs.

**Sample collection and evaluation**

At the end of experimental period, blood was withdrawn from retro-orbital plexus following 12Hr fasting period. The blood samples were allowed to clot at room temperature for 20-25 min and centrifuged for 20 min at 3000 rpm at 4C. The supernatant clear serum thus obtained was stored at -20C until the completion of biochemical analysis. Thereafter, animals were sacrificed by CO₂ overdose and livers were excised immediately and washed with ice-cold saline. Livers were processed further for determination of antioxidant activity in liver tissue homogenate. A portion of excised liver was used for estimation of hepatic lipids [10].

**Biochemical analysis**

All samples were used for following biochemical investigations. The blood serum under this model was analysed for the marker parameters such as Total Cholesterol (TC), High density lipoprotein cholesterol (HDL-C) and Triglycerides (TG). Serum total cholesterol and triglycerides were estimated by enzymatic methods of GHOD-PAP [11] and GPO-Trinder method [12] respectively. Estimation of HDL-C was done by precipitation method [13]. All parameters were analysed by Auto analyser (Erba Chem7) with biochemical kit (Erba diagnostics Mannheim GmbH). Serum concentration of Very low density lipoprotein-Cholesterol (VLDL-C) and Low density lipoprotein-Cholesterol was calculated using Friedewald’s formula [14].

The atherogenic indices calculated were:

- Atherosclerosis Index (A/I) = LDL-C / HDL-C [15]
- Cardiac Risk Ratio (C.R.R)= TC/HDL-C [16]
- Atherogenic Coefficient (AC) = TC-HDL-C/HDL-C [17]

**Antioxidant activity in liver tissue homogenate**

About 1g of liver tissue was homogenised in 10ml of 0.1M phosphate buffer pH 7.4 to form 10% w/v liver tissue homogenates. The prepared liver tissue homogenates were centrifuged at 3500 rpm for 15 minutes and supernatant was used for the determination of various antioxidant parameters like lipid peroxidation levels [18], Reduced glutathione levels [19], and Catalase levels [20].

**Estimation of hepatic lipids**

Hepatic lipids were extracted using method of Folch et al. [1957] [10]. Briefly, 1gm of liver was homogenised in 20ml of solvent mixture (Chloroform: Methanol in ratio 2:1). The homogenate was filtered and washed with 4 ml of saline solution. The mixture was vortexed for few seconds and then centrifuged at 2000 rpm for separation of the two phases. The lower phase was used for estimation of hepatic lipids.

**Statistical analysis**

All results were expressed as means±SEM of 6 rats in each group. The statistical significance between the groups was analysed by using one way ANOVA followed by Dunnett’s multiple comparison test. Significance level was fixed at p<0.01.

**Table 1: Effect of MEBB and EESC on serum lipid profile in hyperlipidemic rats**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>63.38±1</td>
<td>81.04±1</td>
<td>22.07±1</td>
<td>31.11±1</td>
<td>10.21±1</td>
</tr>
<tr>
<td>Control</td>
<td>1.16</td>
<td>2.07</td>
<td>0.80</td>
<td>1.55</td>
<td>0.41</td>
</tr>
<tr>
<td>Disease</td>
<td>152.32</td>
<td>164.09</td>
<td>12.88</td>
<td>107.56</td>
<td>32.79</td>
</tr>
<tr>
<td>Control</td>
<td>2±2.42</td>
<td>2±1.97</td>
<td>0±7.0</td>
<td>2±3.38</td>
<td>0±4.0</td>
</tr>
<tr>
<td>Standard Control</td>
<td>102.08</td>
<td>82.25</td>
<td>33.86</td>
<td>51.99</td>
<td>16.45</td>
</tr>
<tr>
<td>±1.05&quot;</td>
<td>(32.98)↓</td>
<td>(49.88)↓</td>
<td>(162.89)↑</td>
<td>(51.66)↓</td>
<td>(49.88)↓</td>
</tr>
<tr>
<td>MEBB</td>
<td>117.86</td>
<td>96.93</td>
<td>28.32</td>
<td>70.16</td>
<td>19.39</td>
</tr>
<tr>
<td>±1.75&quot;</td>
<td>(22.62)↓</td>
<td>(40.93)↓</td>
<td>(119.88)↑</td>
<td>(34.77)↑</td>
<td>(40.87)↓</td>
</tr>
<tr>
<td>EESC</td>
<td>116.8</td>
<td>105.3</td>
<td>26.92</td>
<td>68.80</td>
<td>20.72</td>
</tr>
<tr>
<td>±1.58&quot;</td>
<td>(23.31)↓</td>
<td>(35.90)↓</td>
<td>(109.0)↑</td>
<td>(35.46)↓</td>
<td>(35.94)↓</td>
</tr>
</tbody>
</table>

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett’s test (p<0.01) Values in parenthesis indicate % increase or decrease in respective parameters, ↓ indicates decrease; ↑ indicates increase.

**RESULTS**

**Biochemical analysis**

In cholesterol suspension induced hypercholesterolemia model, the groups treated with extracts of *B. bambos* (MEBB), *S. chirata* (EESC) and standard drug Atorvastatin demonstrated a significant (p<0.01) decrease in serum levels of total cholesterol (TC), Triglycerides (TG), LDL - Cholesterol (LDL-C), VLDL-Cholesterol (VLDL-C), besides a significant (p<0.01) increase in serum HDL-Cholesterol (HDL-C) levels when compared with disease control group (Table 1). Serum TC levels were reduced by 22.6%, 23.3% and 32.98% by MEBB, EESC and Atorvastatin respectively.
Serum TG levels were reduced by 40.93%, 35.90% and 49.88% by MEBB, EESC and Atorvastatin respectively. Serum LDL-C levels were reduced by 34.77%, 35.46% and 51.66% by MEBB, EESC and Atorvastatin respectively. Serum VLDL-C levels were reduced by 40.87%, 36.94% and 49.83% respectively by MEBB, EESC and Atorvastatin respectively. Serum HDL-C levels were increased by 119.88%, 109.00% and 162.89% by MEBB, EESC and Atorvastatin respectively. The groups treated with MEBB, EESC and standard drug Atorvastatin demonstrated a significant (p<0.01) decrease in Atherosclerosis Index (A.I), Cardiac Risk Ratio (C.R.R) and Atherogenic Coefficient (A.C) when compared with disease control group (Table 2).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>A.I</th>
<th>C.R.R</th>
<th>A.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>1.41±0.04</td>
<td>2.89±0.12</td>
<td>1.89±0.12</td>
</tr>
<tr>
<td>Disease Control</td>
<td>8.38±0.42</td>
<td>12.01±0.70</td>
<td>11.01±0.70</td>
</tr>
<tr>
<td>Standard Control</td>
<td>1.54±0.05*(81.62)</td>
<td>3.02±0.06*(74.85)</td>
<td>2.02±0.06*(81.65)</td>
</tr>
<tr>
<td>MEBB</td>
<td>2.52±0.19*(69.93)</td>
<td>4.21±0.23*(64.95)</td>
<td>3.21±0.23*(70.94)</td>
</tr>
<tr>
<td>EESC</td>
<td>2.62±0.07*(69.45)</td>
<td>4.35±0.10*(63.78)</td>
<td>3.35±0.10*(69.57)</td>
</tr>
</tbody>
</table>

*Values in parenthesis indicate % increase or decrease in respective parameters.

**Table 2: Effect of MEBB and EESC on Atherosclerosis Index (A.I), Cardiac Risk Ratio (C.R.R) and Atherogenic Coefficient (A.C) in hyperlipidemic rats**

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett’s test (p**<0.01) Values in parentheses indicate % decrease in respective parameters.

**Antioxidant activity in liver tissue homogenate**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Hepatic Cholesterol (mg/g)</th>
<th>Hepatic Triglycerides (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>2.33±0.14</td>
<td>6.86±0.12</td>
</tr>
<tr>
<td>Disease Control</td>
<td>7.94±0.68</td>
<td>19.16±0.12</td>
</tr>
<tr>
<td>Standard Control</td>
<td>4.35±0.13*(63.99)</td>
<td>9.67±0.15*(77.15)</td>
</tr>
<tr>
<td>MEBB</td>
<td>4.96±0.05*(53.12)</td>
<td>10.83±0.12*(67.72)</td>
</tr>
<tr>
<td>EESC</td>
<td>5.06±0.07*(51.34)</td>
<td>12.21±0.23*(56.50)</td>
</tr>
</tbody>
</table>

*Values in parenthesis indicate % decrease in respective parameters.

**Table 3: Effect of MEBB and EESC on Hepatic Total Cholesterol and Hepatic Triglyceride levels in hyperlipidemic rats**

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett’s test (p**<0.01) Values in parentheses indicate % decrease in respective parameters.

**Lipid peroxidation, reduced glutathione and Catalase levels in livers of hyperlipidemic rats**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Lipid Peroxidation (µMol/g wt. of wet liver tissue)</th>
<th>Reduced Glutathione (µMol/g wt. of wet liver tissue)</th>
<th>Catalase (µMol/g wt. of wet liver tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>9.2±0.012</td>
<td>2.37±0.054</td>
<td>67.95±0.40</td>
</tr>
<tr>
<td>Disease Control</td>
<td>18.92±0.006</td>
<td>1.93±0.005</td>
<td>41.79±0.34</td>
</tr>
<tr>
<td>Standard Control</td>
<td>10.83±0.012*(83.75)†</td>
<td>2.2±0.009</td>
<td>62.95±0.40</td>
</tr>
<tr>
<td>MEBB</td>
<td>1.38±0.02</td>
<td>(61.36)†</td>
<td>(80.89)†</td>
</tr>
<tr>
<td>EESC</td>
<td>13.19±0.02</td>
<td>2.12±0.01</td>
<td>58.01±0.33</td>
</tr>
</tbody>
</table>

*Values in parenthesis indicate % increase or decrease in respective parameters. † indicates increase.

**Table 4: Effect of MEBB and EESC on Lipid peroxidation, reduced glutathione and Catalase levels in livers of hyperlipidemic rats**

N=6 animals in each group. Values are expressed as mean ± SEM. Values (mean ± SEM) are compared using One way ANOVA followed by Dunnett’s test (p**<0.01). Values in parenthesis indicate % increase or decrease in respective parameters.
useful in reducing the risk of coronary heart disease [26]. Serum HDL-C level may be elevated due to increased activity of lecithin cholesterol acetyl transferase which incorporates free cholesterol, free LDL into HDL-C. During blood circulation, HDL-C mediates the transfer of excess cholesterol from the peripheral cells to liver for its catabolism (reverse cholesterol transport), thereby enhancing the clearance of cholesterol from body [27]. Therefore, increased serum HDL-C levels may prove beneficial in lipid disorders, have a protective role in coronary artery disease [28] and may also act as a cardio protective factor to prevent the gradual initiation of atherosclerotic process [29,30].

Oxidative stress is one of the causative factors that link hyperlipidemia with the pathogenesis of atherosclerosis. This stress results from an imbalance between production of free radicals and effectiveness of antioxidant defense system [31]. The main targets for the free radicals are polyunsaturated fatty acids in membrane of lipids causing lipid peroxidation which may lead to disorganisation of cell structure and function [32]. Lipid peroxidation, a free radical mediated process has been accepted to be one of the primary causes in development of cholesterol induced diseases. Malondialdehyde (MDA), a stable secondary product of lipid peroxidation cascade is widely used as a marker for measurement of lipid peroxidation [33]. Reduced glutathione and Catalase play unique role in cellular defense system by their ability to scavenge free radicals, as such depletion of reduced glutathione and catalase increases vulnerability to free radicals [34,35]. Treatment with MEBB and EESC significantly (p<0.01) decreased hepatic MDA levels while increasing hepatic reduced glutathione and catalase levels. Administration of MEBB and EESC showed significant (p<0.01) antioxidant activity in liver tissue homogenate and may have protective role against oxidative stress induced atherosclerosis.

Atherogenic indices are powerful indicators of the risk of development of heart disease: higher the value, higher the risk of development of cardiovascular disease and vice versa [36,37]. Treatment with MEBB and EESC lowered atherogenic indices significantly (p<0.01) thus decreasing the risk of development of cardiovascular disease.

Our results suggest that MEBB and EESC have effective hypcholesterolemic action together with antioxidant effect. Detailed studies are needed to postulate the possible mechanism(s) of action.

REFERENCES