Chapter 5

Antihypocholesterolemic and Antioxidant Effect of AqMS
CHAPTER 5
ANTIHYPERTROCHOLESTEROLEMIC AND ANTIOXIDANT EFFECT OF AQUEOUS EXTRACT OF CENTRAL STEM OF MUSA SAPIENTUM

5.1 INTRODUCTION
Coronary artery disease (CAD) is one of the most important cause of death all over the world (Tryoler, 1999). Hypercholesterolemia contributes significantly in the manifestation and development of atherosclerosis and coronary artery disease (Yokozawa et al, 2003). Although several factors such as diet rich in saturated fats, cholesterol age, family history, hypertension, life style etc. play significant role in causing the CAD, the high levels of cholesterol particulary LDL-cholesterol is mainly responsible for the onset of CAD (Farias et al, 1996). Many plants have been reported to have potential to decrease lipids including cholesterol in body viz. Terminalia arjuna, Ficus bangalensis etc (Gupta et al, 2001; Shukla et al, 2004). As preliminary studies demonstrates AqMS to have hypocholesterolemic effect in diabetic rats, this study was planned to evaluate the antihypercholesterolemic effect of central stem of Musa sapientum in rats, made hypercholesterolemic by feeding cholesterol As hypercholesterolemia increased oxidative stress, studies were also conducted to evaluate antioxidant effect of AqMS.

5.2 RESULTS
5.2.1 Assessment of Hypocholesterolemic Effect of Aqueous Extract of central stem of Musa Sapientum
Treatment regime- Animals were divided into four groups of six animals each and treated for 6 weeks as follows:- Group I-Healthy control rats fed with saline (0.5 ml/kg); Group II- Vehicle control rats fed with vehicle soya oil (1.5 mg/kg); Group III- Rats fed with cholesterol suspended in soya oil (100mg /kg) (Hypercholesterolemic Control group); Group IV Rats fed with cholesterol suspended in soya oil (100mg/kg) + Aqueous extract of central stem of Musa sapientum (AqMS) at a dose of 50mg/kg.

Table 5.1 shows the effect of treatment with AqMS on serum lipid profile parameters. In hypercholesterolemic rats, which received cholesterol suspended in soya oil rise in
cholesterol level was 184%. Serum total cholesterol also increased in soya oil fed group by 88%, whereas in group IV, treatment with AqMS at a dose of 50 mg/kg in addition to cholesterol suspended in soya oil, there was significant fall in levels of serum total cholesterol compared to rats group III which received cholesterol suspended in oil alone.

Triacylglycerol increased in soya oil fed group & hypercholesterolemic rats which received cholesterol suspended in soya oil but in rats in which AqMS was co-administered along with cholesterol, there was significant fall in levels of triacylglycerol compared to hypercholesterolemic rats and vehicle treated rats.

HDL-C levels decreased in hypercholesterolemic rats compared to healthy control rats. AqMS treated group showed significant rise in levels of HDL-C (p<0.01). There was no significant change in HDL-C in soya oil fed group.

LDL+VLDL-C increased in both soya oil fed group & hypercholesterolemic group of rats, rise in these parameters was more in hypercholesterolemic group which received cholesterol in soya oil (170%). There was significant fall in LDL+VLDL-C in AqMS treated group IV compare to group III. (p<0.01) Levels of TC, TAG and LDL+VLDL-C were still higher in AqMS treated group compared to healthy control, as AqMS treated group were also given cholesterol in addition to AqMS.

**Table 5.1: Effect of AqMS treatment (50mg/kg) on serum lipid profile in cholesterol fed rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (Healthy control rats)</th>
<th>Group II (Vehicle control)</th>
<th>Group III (Hypercholesterolemic Rats)</th>
<th>Group IV (Hypercholesterolemic +AqMS (50mg/kg))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cholesterol (mg/dl))</td>
<td>54± 1.2</td>
<td>101± 2.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>154 ± 2.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>77± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>74± 1.4</td>
<td>126± 1.8&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>128± 2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>89±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>32± 0.8</td>
<td>27.4± 0.9&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>23± 1.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>28±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL+VLDL-C (mg/dl)</td>
<td>17± 1.6</td>
<td>74± 1.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>104±1.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>31±1.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M of six rats in group;
<sup>a</sup>p< 0.01 hypercholesterolemic rats treated with AqMS compared to hypercholesterolemic control rats
<sup>ab</sup>p< 0.001 Hypercholesterolemic rats compared to healthy control rats
<sup>abc</sup>p< 0.01 vehicle control rats compared to healthy control rats
5.2.2. Effect of treatment with Aqueous extract of central stem of *Musa sapientum* (50mg/kg) on Atherogenic index (LDL+VLDL-C/ HDL-C)

*Figure 5.1* shows effect of AqMS treatment on atherogenic index. Atherogenic index increased in vehicle treated group and cholesterol fed group compared to healthy control. Treatment with AqMS resulted in significant decrease in atherogenic index.

5.2.3. Effect of Aqueous extract of stem of *Musa sapientum* (50mg/kg) on antioxidant parameters of blood

Effect of AqMS treatment on MDA, GSH, SOD & CAT in blood is shown in *Table 5.2*. Feeding cholesterol suspended in oil increased MDA in hypercholesterolemic rats. Treatment with AqMS resulted in significant fall in levels of MDA (p<0.01). Levels of GSH decreased in hypercholesterolemic rats. Rats which received AqMS treatment showed 72% increase in levels of GSH. Enzyme SOD & CAT decreased in hypercholesterolemic rats (47%, 64% fall respectively), while treated group showed significant rise in level of SOD & CAT (p<0.01). Rats in soya oil fed group which were given only vehicle (soya oil) showed significantly fall in levels of SOD, rise in MDA and no appreciable effect on GSH & CAT.

**Table 5.2: Effect of Aqueous extract of central stem of *Musa sapientum* (AqMS) at a dose of 50mg/kg on lipid peroxidation and antioxidant parameters in blood**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (Healthy control rats)</th>
<th>Group II (Vehicle control)</th>
<th>Group III (Hypercholesterolemic control rats)</th>
<th>Group IV (Hypercholesterolemic rats treated with AqMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (nmol/ ml)</td>
<td>3.4±0.2</td>
<td>4.1±0.2abc</td>
<td>6.1±0.16ab</td>
<td>3.4±0.12a</td>
</tr>
<tr>
<td>Reduced glutathione (mg/gHb)</td>
<td>2.2±0.2</td>
<td>1.9±0.08abc</td>
<td>1.1±0.07ab</td>
<td>1.9±0.07a</td>
</tr>
<tr>
<td>Superoxide dismutase (u/gmHb)</td>
<td>2191±83</td>
<td>1805±45abc</td>
<td>1144±49ab</td>
<td>1652±60a</td>
</tr>
<tr>
<td>Catalase (u/gmHb)</td>
<td>2.5±0.16</td>
<td>2.1±0.04abc</td>
<td>0.9±0.04ab</td>
<td>1.9±0.08a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M of six rats in group;

*ap< 0.01 hypercholesterolemic rats treated with AqMS compared to hypercholesterolemic control rats
abp< 0.001 Hypercholesterolemic rats compared to healthy control rats
abc p< 0.01 vehicle control rats compared to healthy control rats*
5.2.4. Effect of Aqueous extract of central stem of *Musa sapientum* (50mg/kg) on lipid peroxidation and antioxidant parameters in liver

*Table 5.3* showed the effect of AqMS treatment on MDA, GSH, SOD and CAT in liver tissues. MDA increased in liver of soya oil fed group & hypercholesterolemic rats, which received cholesterol suspended in oil. Treatment with AqMS resulted in significant fall in level of MDA in AqMS treated rat. Levels of SOD, CAT and GSH significantly decreased in hypercholesterolemic rat which received cholesterol suspended in oil. Treatment with AqMS along with cholesterol in soya oil resulted in significant rise in the levels of these parameters. In vehicle treated group levels of GSH, SOD decreased, while there was no change in values of GSH and CAT.

*Table 5.3*: Effect of Aqueous extract of central stem of *Musa sapientum* (AqMS) at a dose of 50 mg/kg on lipid peroxidation and antioxidant parameters in liver of hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (Healthy control rats)</th>
<th>Group II (Vehicle control)</th>
<th>Group III (Hypercholesterolemic control Rats)</th>
<th>Group IV (Hypercholesterolemic rats treated with AqMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (nmol/mg protein)</td>
<td>1.4±0.2</td>
<td>3.3±0.5</td>
<td>4.4±0.6</td>
<td>2.2±0.6</td>
</tr>
<tr>
<td>Reduced Glutathione (μmoles/gm tissue)</td>
<td>9.5±0.7</td>
<td>8.3±0.7</td>
<td>2.2±0.3</td>
<td>5.9±0.9</td>
</tr>
<tr>
<td>Super oxidizedismutase (u/mg protein)</td>
<td>2086±250</td>
<td>1933±151</td>
<td>1244±220</td>
<td>1652±144</td>
</tr>
<tr>
<td>Catalase (nmol H₂O₂ decomposed /mg of protein /60 s)</td>
<td>185±20</td>
<td>180±30</td>
<td>117±17</td>
<td>171±20</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M of six rats in group;

- *p* < 0.01 hypercholesterolemic rats treated with AqMS compared to hypercholesterolemic control rats
- *p* < 0.001 Hypercholesterolemic rats compared to healthy control rats
- *p* < 0.01 vehicle control rats compared to healthy control rats
Values are mean ± S.E.M of six rats in group;

\( ^a \)p < 0.01 hypercholesterolemic rats treated with AqMS compared to hypercholesterolemic control rats

\( ^b \)p < 0.001 Hypercholesterolemic rats compared to healthy control rats

\( ^c \)p < 0.01 vehicle control rats compared to healthy control rats

Figure 5.1: Effect of treatment with aqueous extract of central stem of *Musa sapientum* (AqMS) (50mg/kg) on Atherogenic index (LDL+VLDL-C/HDL-C)
5.3. DISCUSSION

Therapeutic relevance of natural products extracts is of paramount importance (Patwardhan et al, 2005). The present study has demonstrated that aqueous extract of central stem of *Musa sapientum* has significant antihypercholesterolemic and antioxidant effect. It was effective at a low dose of 50 mg/kg, when co-administered with cholesterol suspended in oil. AqMS treatment produced significant fall in total cholesterol, LDL+VLDL-cholesterol, triacylglycerol by 48%, 60 % & 29% respectively and increased HDL-C by 21%. Compared to untreated hypercholesterolemic rats, atherogenic index decreased significantly indicating a reduction in harmful lipid pool relative to favourable one.

Hypercholesterolemia can increase production of oxygen free radicals, which may results in lipid peroxidation leading to increase in formation of malondialdehyde (MDA) (Gupta et al, 2001; Shukla et al, 2004). Lipid peroxide were measured as the thiobarbituric acid reactive substances. An increase in MDA indicating decrease in antioxidant status in cholesterol fed animals is observed in this study, which is in agreement with other studies (Küskü et al, 2010; Ahmed et al, 2001). Hypercholesterolemia induces oxidative stress. Enhanced oxidative stress may depress antioxidant defences (Dikshit et al, 2011; Shukla et al, 2004). In the present study we observed decreased activities of antioxidant enzymes SOD and Catalase in erythrocytes and liver of rats maintained on high cholesterol diet compared to healthy controls. Simultaneous treatment of cholesterol fed rats with AqMS increased activity of SOD and catalase significantly. (p<0.01) A decrease in rise of MDA when AqMS was co-administered along with cholesterol suggested that AqMS can decrease lipid peroxidation and improve antioxidant status. More over AqMS treatment prevented decrease in GSH, which was observed in rats receiving cholesterol. This beneficial effect of AqMS treatment was seen not only in blood but also in liver tissue.

Important observation of the present study is that feeding soya oil which was used as a vehicle in this experiment could increase TC, TAG, LDL+VLDL-C. Feeding rats with soya oil has no effect on HDL-C. In one study, where corn oil was used as a vehicle, feeding corn oil also resulted in increase of TC, TAG & LDL+VLDL-C (Gosain et al, 2010). Rats receiving soya oil showed increased MDA and decreased
SOD levels in blood & tissue but it has no significant effect on levels of ‘GSH’ and antioxidant enzyme ‘catalase’. Results of the present study show that soya oil alone can increase serum total cholesterol, TAG and LDL+VLDL-C and induce oxidative stress, which is evident from increased levels of MDA and decreased levels of antioxidant enzyme SOD, although changes in lipid profile and antioxidant parameters are less profound in vehicle treated group. Result presented in this chapter clearly demonstrated that AqMS has significant hypocholesterolemic and antioxidant effect.