Chapter 7

Effect of tender coconut water in high fructose fed hypertensive rats compared to antihypertensive drug, amlodipine

A variety of therapies is available for treatment of hypertensives with metabolic complications. Lifestyle modification is considered to be the initial approach. Although diuretics and beta blockers have a proven record in reducing morbidity and mortality, they may have adverse effects on glucose, insulin and lipids and should be used with caution in hypertensives with metabolic risks. Calcium antagonists have no adverse effects on lipid and glucose (Canan et al., 2004). Pharmacological management of hypertension is generally directed to the normalization of altered haemodynamic parameters (Minz et al., 1992).

Among antihypertensive therapies, the lipophilic dihydropyridine type calcium channel blocker amlodipine besylate (AML) is a very well tolerated agent with an established record of safety and effectiveness for the treatment of hypertension (Murdoch and Heel, 1991). Amlodipine is chemically described as (R.S.) 3-ethyl 5-methyl-2-(2 aminoethoxy methyl)-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine carboxylate. Its empirical formula is C_{20}H_{25}C_{1}N_{2}O_{5}. Studies have reported that amlodipine possesses antioxidant (Mason et al., 1999), cardioprotective (Luccheri et al., 1991), hypoinsulinemic and antihypertensive properties (de la sierra Alejandro, 2007;
M de courten et al, 1993). Since tender coconut water mediates lowering of systolic and diastolic blood pressure, lipids and lipid peroxides in fructose fed rats, we compared the hypolipidemic and antioxidant effect of tender coconut water with antihypertensive drug Amlodipine in high fructose fed hypertensive rats.

7.1 Materials and Methods

Experimental Groups

Male albino rats (Sprague Dawley strain) weighing 160-180g, were used for the study. The rats were divided into 4 groups of six each and fed the following diet.

Group 1 Control rats
Group 2 High fructose fed (hypertensive) rats
Group 3 High fructose fed (hypertensive) rats+ TCW (4ml/100 g body weight)
Group 4 High fructose fed (hypertensive) rats+Amlodipine (5 mg/Kg body weight)

Rats were fed the respective diet and maintained for 5 weeks. Group 1 received the standard starch diet. Groups 2, 3 and 4 received high fructose diet. After 2 weeks rats of group 3 received TCW (4 ml/100 g body weight) and group 4 received Amlodipine (5 mg/kg body weight) dissolved in water by gastric intubation. All other experimental conditions were the same as described in chapter 3. At the end of experimental period they were sacrificed and blood and tissues were collected in ice cold containers.
7.2 Results

Following biochemical parameters were studied:

7.2.1 Systolic and diastolic blood pressure

Systolic and diastolic blood pressure significantly increased in rats fed high fructose diet. On the other hand, supplementation with tender coconut water or amlodipine significantly reduced the blood pressure (Fig. 22 and 23).

Fig. 22 Effect of tender coconut water and amlodipine on Systolic blood pressure

Values are mean ± SD for six rats. P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.
Fig 23. Effect of tender coconut water and amlodipine on diastolic blood pressure

Values are mean ± SD for six rats.

P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.2 Concentration of total cholesterol in serum

Rats fed high fructose diet showed significant increase in concentration of serum total cholesterol. On the other hand, the total cholesterol significantly lowered in rats supplemented with tender coconut water or amlodipine (Fig. 24).
Values are mean ± SD for six rats. 
P<0.05, * indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.3 Concentration of triglycerides in serum

Rats fed high fructose diet showed significant increase in concentration of serum triglycerides. On the other hand, triglycerides significantly lowered in rats supplemented with tender coconut water or amlodipine (Fig. 25).
Values are mean ± SD for six rats.
P<0.05, * indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.4 Concentration of cholesterol in tissues

Concentration of cholesterol in liver, heart and kidney were higher in rats fed with high fructose diet compared to normal rats. Rats fed with tender coconut water or amlodipine showed significant lower concentration of these tissue cholesterol compared to fructose fed hypertensive rats (Fig. 26).
Fig. 26  Concentration of cholesterol in tissues

Values are mean ± SD for six rats. 
P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.5 Concentration of triglyceride in tissues

Concentration of triglycerides in liver, heart and kidney were higher in rats fed high fructose diet compared to normal rats. Rats fed with tender coconut water or amlodipine showed significant lower concentration of triglycerides compared to fructose fed hypertensive rats (Fig. 27).
Values are mean ± SD for six rats. P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.6 Concentration of malondialdehyde in tissues

Concentration of MDA (TBARS) in liver, heart and kidney were higher in rats fed with high fructose fed diet compared to normal rats. Tender coconut water or amlodipine supplementation in hypertensive rats high fructose diet decreased the concentration of this lipid peroxide in tissues compared to hypertensive rats fed with high fructose diet (Fig. 28).
Fig. 28  Concentration of malondialdehyde in tissues

Malondialdehyde

Values are mean ± SD for six rats. P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.7 Activity of superoxide dismutase in tissues

The activity of superoxide dismutase (SOD) was significantly lower in liver, heart and kidney of high fructose fed rats compared to normal rats. Administration of tender coconut water and amlodipine resulted in increased activity of the enzyme (Fig. 29).
Fig. 29 Activity of Superoxide dismutase* in tissues

![Superoxide dismutase graph]

Values are mean ± SD for six rats.
*One unit is defined as the enzyme concentration required to inhibit the optical density at 560 nm of chromogen produced by 50% in one minute.
P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.8 Activity of catalase in tissues

The activity of catalase was significantly lower in liver, heart and kidney of high fructose fed rats when compared to control rats. Administration of tender coconut water and amlodipine resulted in increased activity of the enzyme (Fig. 30).
Fig. 30 Activity of catalase * in tissues

Values are mean ± SD for six rats.
* One unit is defined as the velocity constant per second.
P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

7.2.9 Activities of SGOT, SGPT and ALP in serum

Activities of serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were significantly higher in rats with fed high fructose diet compared to normal rats. Administration of tender coconut water and amlodipine decreased the activities of these enzymes compared to high fructose fed control rats (Table 21).
### Table 21 Activities of SGOT, SGPT and ALP in serum

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (KA units/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.1±1.64b</td>
<td>23.41±3.12b</td>
<td>10.97±0.84b</td>
</tr>
<tr>
<td>2</td>
<td>49±2.61a</td>
<td>35.71±1.35a</td>
<td>13.5±1.62a</td>
</tr>
<tr>
<td>3</td>
<td>28.3±2.43b</td>
<td>26.71±0.91b</td>
<td>10.1±0.13b</td>
</tr>
<tr>
<td>4</td>
<td>26.95±0.77b</td>
<td>25.31±0.65b</td>
<td>9.83±0.27b</td>
</tr>
<tr>
<td>F ratio</td>
<td>204.34</td>
<td>56.35</td>
<td>20.54</td>
</tr>
</tbody>
</table>

Values are mean ± SD for six rats. P<0.05, a indicates that the results are significantly different from group 1, b indicates that the results are significantly different from group 2, c indicates that the results are significantly different from group 4.

### 7.2.10 Histopathological studies

Histopathological studies of liver and aorta showed fatty infiltration in fructose fed hypertensive rats which was reversed on treatment with tender coconut water and amlodipine (Plate 5 and 6).
Plate 5: Light microscopic appearance of the liver sections stained with Hematoxylin-Eosin (x 100)

1. Control
The liver architecture is normal with cords of hepatocytes with normal cytoplasm and central nuclei. There are no inflammatory cells in the portal tract nor in the parenchyma. There are no signs of cellular damage.

2. Fructose fed hypertensive rats
Portal inflammation and inflammatory fatty infiltration is noticed.

3. Fructose fed hypertensive rats + TCW
No hepatocellular damage and inflammatory infiltration. Lower lipid accumulation (LA).

4. Fructose fed hypertensive rats + Amlodipine
No hepatocellular damage, fatty infiltration and lipid accumulation.
Plate 6: Light microscopic appearance of the aorta sections stained with Hematoxylin-Eosin (x 400)

1. **Control**
   Structure of normal aorta consists of Intima (IA) - Innermost layer lined by endothelial cells, Media (MA)- contains elastic fibers (EF) and Adventitia-consist of fibrous outer covering. No abnormal features.

2. **Fructose fed hypertensive rats**
   In hypertensive rats thickness of aorta is increased. Space between the layers of aorta is increased. It indicates the lipid accumulation (LA) between the layers. Deposits of mucopolysaccharides in the elastic fibers.

3. **Fructose fed hypertensive rats + TCW**
   Thickness of aorta is reversed and medial hypertrophy is reversed. No lipid accumulation. No deposits of mucopolysaccharides.

4. **Fructose fed hypertensive rats + Amlodipine**
   Thickness of aorta is reversed and medial hypertrophy is reversed. Lower lipid accumulation.
7.3 Discussion

The results obtained indicate that both tender coconut water and amlodipine lowered the systolic and diastolic blood pressure in rats fed with high fructose diet. Amlodipine’s ability to block calcium channels in smooth muscle produces peripheral vasodilation resulting in decrease in both systolic and diastolic blood pressure in hypertensives (Atarashi et al, 1986). In addition tender coconut water and amlodipine have comparable hypolipidemic action in rats fed with high fructose diet. On supplementation of tender coconut water or amlodipine, total cholesterol in serum and tissues were lower. Amlodipine is reported to reduce the cholesterol accumulation (Kabaroglu et al, 2003). Serum and tissue triglycerides was also lowered on treatment with tender coconut water and amlodipine.

The lipid lowering effect of rats which received high fructose diet supplemented with tender coconut water or amlodipine would be the results of various mechanisms such as lowering intestinal absorption of dietary lipids, reduced biosynthesis or increased catabolism of cholesterol and fatty acids in liver. The decrease in serum triglycerides in rats fed with tender coconut water or amlodipine partly be due to increased activity of LPL and decreased lipogenesis (Sandhya and Rajamohan, 2006).

Feeding high fructose diet increased the activities of SGPT, SGOT and ALP in serum while supplementation of tender coconut water led to decreased activity of these enzymes. This indicates that fatty liver caused by high fructose
diet would significantly reduce by tender coconut water. Amlodipine treatment also prevents the fatty liver as supported by other studies (Zvi et al, 2007).

In addition to the hypolipidemic effect, feeding tender coconut water and amlodipine significantly reduced the lipid peroxidation evident from decreased tissue lipid peroxide content via MDA. Amlodipine is reported to inhibit lipid peroxide formation (Preston et al, 1999). Activities of antioxidant enzymes, superoxide dismutase and catalase were seen decreased in high fructose fed hypertensive rats. On the other hand, supplementation of tender coconut and amlodipine significantly increased the enzyme activities. Amlodipine is reported to upregulate the activity of SOD and prevent the decrease in catalase (Mason et al, 2003; Kabaroglu et al, 2003). The decrease in SOD may be associated with a decrease in NO levels (Mutaf et al, 2004).

From the results it is clear that the antihypertensive, hypolipidemic and antioxidant effects were similar in rats fed with tender coconut water and amlodipine.