Chapter 8

Effect of tender coconut water in high fructose fed hypertensive rats with and without L-NAME

In the previous studies it was shown that tender coconut water has significant antihypertensive, hypolipidemic, antioxidant and hypoinsulinemic effects. Feeding TCW was found to be effective as antihypertensive drug, amlodipine in reducing the blood pressure, blood lipids and enhancing antioxidant property. It was also discussed that these effects of TCW are due to the presence of several biologically active components viz L-Arginine, Vitamin C, Calcium, Magnesium and Potassium. It was previously discussed that, among these active components, L-Arginine is the most important factor which influences blood lipids, glucose and blood pressure mediated via the formation of nitric oxide by the enzyme nitric oxide synthase which has been reported to have vasodilator effect. L-Arginine has been reported to regulate the vascular tone and haemostasis (Udvardy et al, 1997). L-Arginine seems to be a regular element in the modification of fibrinogenolysis in a wide variety of the new generation antithrombotic agents (Imura et al, 1992; Topol, 1995).

The vascular endothelium is responsible for the production of several vasoactive substances, of the most vital of which is nitric oxide, a potent vasodilator synthesized from L-Arginine by the enzyme nitric oxide synthase (NOS) (Loscalzo, 2000). Several studies have shown that endothelial synthesis of nitric oxide may be impaired in hypertension. The inhibition of nitric oxide
synthase by L-NAME (L-\(\text{N}^G\)-Nitroarginine methyl ester) seems to be involved in lipid metabolism alterations (Khedara et al, 1996). NOS inhibited by Asymmetric Dimethyl Arginine (ADMA) is known to elevate hypercholesterolemia and mononuclear cell adhesion (Adams et al, 1997).

Increased levels of haemostatic factors and genetic mutations of proteins involved in coagulation may also play a role in blood pressure.

Thus the present study was carried out using L-NAME to evaluate the role of L-Arginine and nitric oxide availability on lipid levels and coagulation factors in fructose induced hypertension.

8.1 Materials and Methods

Experimental Groups

Male albino rats (Sprague Dawley strain) weighing 150-170g, 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 (4 ml/100g body weight)

Group 4 High fructose fed (hypertensive) rats+ L-NAME (0.5 mg /kg/day)

Group 5 High fructose fed (hypertensive) rats+ TCW + L-NAME

Rats were fed the respective diet and maintained for 5 weeks. Rats of group 1 were fed standard starch diet. Groups 2, 3, 4 and 5 received high fructose diet. After 2 weeks, rats of group 3 and 5 were given TCW (4 ml/100
g body weight) by gastric intubation. Group 4 and 5 received L-NAME (0.5 mg kg\textsuperscript{-1} day\textsuperscript{-1}) dissolved in water given by gastric intubation after 2 weeks. 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. Before killing, 24 hours urine samples were collected thrice from rats of each group in metabolic cages, pooled and used for estimation of urinary nitrite.

8.2 Results

Following biochemical parameters were studied:

8.2.1 Systolic and Diastolic blood pressure

Systolic and diastolic blood pressure were higher in hypertensive control rats compared to normal rats. Feeding TCW to hypertensive rats significantly reduced the blood pressure. On the other hand, TCW fed rats treated with L-NAME showed no significant beneficial effects compared to those fed TCW alone (Fig. 31 and 32).
**Fig. 31** Effect of tender coconut water on systolic blood pressure compared to L-NAME and without L-NAME in hypertensive rats

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 5.

**Fig. 32** Effect of tender coconut water on diastolic blood pressure compared to L-NAME and without L-NAME in hypertensive rats

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 5.
8.2.2 Concentration of serum total cholesterol

Concentration of serum total cholesterol was higher in hypertensive control rats when compared to normal rats. Feeding TCW to hypertensive rats showed significant decrease in serum total cholesterol when compared to hypertensive control rats. On the other hand, TCW fed rats treated with L-NAME showed no significant beneficial effects compared to those fed TCW alone (Fig. 33).

![Concentration of serum total cholesterol](image)

Values are mean ± 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 5.

8.2.3 Concentration of triglycerides in serum

Concentration of triglycerides in the serum was higher in hypertensive groups when compared to normal rats. Feeding TCW in hypertensive rats resulted in significant decrease in the concentration of triglycerides in serum when compared to hypertensive rats. While TCW fed rats treated with L-
NAME showed no significant beneficial effect compared to those fed TCW alone (Fig. 34).

**Fig. 34 Concentration of serum triglyceride**

<table>
<thead>
<tr>
<th>Triglyceride (mg/dl)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td></td>
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<td></td>
<td></td>
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<td>14</td>
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<td>12</td>
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<td>10</td>
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<td>8</td>
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<td>6</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>b</td>
<td>a</td>
<td>b,c</td>
<td>a</td>
<td>a</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 5.

8.2.4 Activities of nitric oxide synthase and arginase in liver

Activity of nitric oxide synthase in the liver was decreased significantly in hypertensive rats while the activity of arginase increased in these groups when compared to normal rats. Feeding TCW in hypertensive rats showed significant increase in the activity of nitric oxide synthase and decreased activity of arginase in the liver when compared with hypertensive control rats. While TCW fed rats treated with L-NAME showed no significant beneficial effect compared to those fed TCW alone (Table 22).
### Table 22 Activities of nitric oxide synthase (Units/mg protein) and arginase (U/g wet weight) in liver

<table>
<thead>
<tr>
<th>Groups</th>
<th>Nitric oxide synthase</th>
<th>Arginase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.824±0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>576.1 ±8.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.438±0.049&lt;sup&gt;a&lt;/sup&gt;</td>
<td>862.5±16.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.012±0.035&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>571.75±5.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.337±0.0039&lt;sup&gt;a&lt;/sup&gt;</td>
<td>896.75±20.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>0.652±0.059&lt;sup&gt;a&lt;/sup&gt;</td>
<td>681.25±5.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F ratio</td>
<td>209.25</td>
<td>597.53 &lt;sup&gt;&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD for six rats.
P<0.05, <sup>a</sup> indicates that the results are significantly different from group 1, <sup>b</sup> indicates that the results are significantly different from group 2, <sup>c</sup> indicates that the results are significantly different from group 5.

#### 8.2.5 Concentration of plasma and liver L-Arginine and Urinary nitrite

Urinary nitrite concentration and L-Arginine content in plasma and liver were increased in rats fed TCW when compared to hypertensive control rats. While tender coconut water fed rats treated with L-NAME showed no significant beneficial effect compared to those fed tender coconut water alone (Table 23).
Table 23 Concentration of L-Arginine in plasma and liver and urinary nitrite

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma arginine (µ mol/ml)</th>
<th>Liver arginine (mg/g tissue)</th>
<th>Urinary nitrite (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.129±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.037±0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.8±2.836&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.09±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0195±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.87±1.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.185±0.013&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.048±0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.65±0.750&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.062±0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.017±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.3±0.489&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>0.163±0.013&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.044±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.2±0.408&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F ratio  | 103.398  | 74.835  | 11.478  

Values are mean ± SD for six rats. 
P<0.05, <sup>a</sup> indicates that the results are significantly different from group 1, <sup>b</sup> indicates that the results are significantly different from group 2, <sup>c</sup> indicates that the results are significantly different from group 5.

8.2.6 Blood cell count

The White Blood Cell (WBC) and platelet count were significantly higher in hypertensive rats compared to control rats. Feeding of TCW to hypertensive rats decreased the levels of WBC and platelet. On the other hand TCW fed rats treated with L-NAME showed no significant beneficial effects compared to TCW alone fed groups (Table 24).
Table 24 Levels of WBC and Platelet

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (× 10^3/cmm)</th>
<th>Platelet (× 10^9/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.74±1.93^b</td>
<td>2.057±0.009^b</td>
</tr>
<tr>
<td>2</td>
<td>9.93±0.263^a</td>
<td>3.43±0.221^a</td>
</tr>
<tr>
<td>3</td>
<td>7.67±0.411^b,c</td>
<td>2.15±0.0191^b,c</td>
</tr>
<tr>
<td>4</td>
<td>10.5±0.183^a</td>
<td>3.7±0.129^a</td>
</tr>
<tr>
<td>5</td>
<td>9.28±0.176^a</td>
<td>3.05±0.129^a</td>
</tr>
<tr>
<td>F ratio</td>
<td>5.864</td>
<td>128.13</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 5.

8.2.7 Concentration of fibrinogen, fibrin and prothrombin time

Hypertensive rats showed a significant increase in the levels of fibrinogen and fibrin and a significant decrease in prothrombin time, compared to control rats. Feeding of TCW in hypertensive rats decreased the level of fibrinogen, fibrin and increased prothrombin time compared to hypertensive control rats. On the other hand, TCW fed rats treated with L-NAME showed no significant beneficial effects compared to TCW alone fed groups (Table 25).
8.2.8 Concentration of factor V

The concentration of factor V was significantly higher in hypertensive rats compared to normal rats. Treatment with tender coconut water restored the level compared to hypertensive control rats. On the other hand, TCW fed rats treated with L-NAME showed no significant beneficial effects compared to tender coconut water alone fed groups (Table 26).
# Table 26 Concentration of factor V in plasma

<table>
<thead>
<tr>
<th>Groups</th>
<th>Factor V (second)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.1±2.174b</td>
</tr>
<tr>
<td>2</td>
<td>34.7±0.275a</td>
</tr>
<tr>
<td>3</td>
<td>29.2±0.607b,c</td>
</tr>
<tr>
<td>4</td>
<td>36.0±0.454a</td>
</tr>
<tr>
<td>5</td>
<td>32.05±0.835a</td>
</tr>
<tr>
<td><strong>F ratio</strong></td>
<td><strong>45.49</strong></td>
</tr>
</tbody>
</table>

Values are mean ± SD for six rats.
*decreased clotting time indicates increased concentration.
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 5.

## 8.2.9 Histopathological studies

Histopathological studies of liver and aorta shows degenerative changes and fatty infiltration in L-NAME treated rats compared to group without L-NAME. On other hand, fatty infiltration and degenerative changes were minimised in tender coconut water treated rats (Plate 7 and 8).
Plate 7: 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 + L-NAME**
   Hepatocellular damage and cytoplasmic vacuolization. Necrotic areas with inflammatory cells in addition to intralobular infiltration.

5. **Fructose fed hypertensive rats + TCW + L-NAME**
   Hepatocellular damage and cytoplasmic vacuolization is minimized.
   No intralobular infiltration.
Plate 8: 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- fibrous outer covering. No abnormal features.

1. **Fructose fed hypertensive rats**

In hypertensive rats thickness of aorta is increased, 5 layers. 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 and medial hypertrophy is reversed. Layers same as that of control. Elastic fibers appears normal. Lower lipid accumulation.

4. **Fructose fed hypertensive rats + L-NAME**

The thickness of aorta is increased, 7 layers. Elastic fibers appears to be wavy. Lipid accumulation and deposits of mucopolysaccharide is increased.

5. **Fructose fed hypertensive rats + TCW+ L-NAME**

The thickness of aorta is reduced, 5 layers. Lower lipid accumulation and deposits of mucopolysaccharides.
8.3 Discussion

The present study was carried out using L-NAME (NOS inhibitor) to evaluate the role of L-Arginine and nitric oxide availability on lipid levels and coagulation factors in fructose fed rats given tender coconut water. The systolic and diastolic blood pressure significantly increased in L-NAME treated rats in comparison with rats fed without L-NAME group as supported by other studies (Tolins et al., 1990; Paulis et al., 2008). TCW administration significantly reduced the blood pressure which may be partly due to the presence of L-Arginine. L-Arginine as a precursor for nitric oxide, an important signalling molecule in cardiovascular system has been reported to augment vascular dilation. The vasodilator action of L-Arginine may lead to hypotension (Nakaki et al., 1990; Aydin et al., 2002).

L-NAME administration showed significantly higher levels of serum cholesterol and triglycerides while in those fed without L-NAME the levels of serum cholesterol and triglycerides were lower. Low nitric oxide level appears to be associated with hypertension in rats (Cylwik, 2004). Our data is in accordance with observation that, reduced nitric oxide availability increases total cholesterol and triglyceride (Khedara et al., 1996; Goto et al., 1999). Reduced activity of endothelial cell NOS in presence of LDL cholesterol is reported to be overcome by L-Arginine (Pritchard et al., 1995). L-Arginine is reported to exert antiatherosclerotic effects (Schachinger et al., 2000).
Activity of nitric oxide synthase and urinary nitrite was significantly lower in L-NAME treated group than those fed without L-NAME. Urinary nitrite excretion is an indicator of nitric oxide formation in vivo during oral administration of L-Arginine or L-NAME in rats (Boger et al, 1996). Dietary nitrite is reported to ameliorate degenerative changes in L – NAME induced hypertensive rats (Kobayashi et al, 2010). Acute administration of L-Arginine increases urinary excretion of nitrate (Cooke et al, 1991). L-Arginine and vitamin C is reported to mediate the protection of tetrahydrobiopterin and restoration of eNOS activity (Wever et al, 1998).

Treatment with L-NAME increased the arginase activity while those fed without L-NAME reversed this effect. Similar studies have been reported by others (Berkowitz et al, 2003). Increase in Arginase activity has been implicated in endothelial dysfunction (Ming and Barandier, 2004). The availability of L-Arginine can be a rate limiting factor for cellular NO production by NOS. Arginase competes with NOS for L-Arginine as the common substrate. Increased arginase activity has been linked to low NO levels and an inhibition of Arginase activity has been reported to improve endothelial dependent vasorelaxation (Oliver et al, 2008).

In L-NAME treated rats the fibrinogen levels and factor V increased and prothrombin time was found reduced in comparison with rats fed without L-NAME group, where the levels were found reversed. In the present study fructose administration resulted in abnormal blood clotting and was evident
from the higher levels of coagulation factors namely fibrin, fibrinogen, factor V and decreased levels of prothrombin time. Administration of TCW prolonged PT which indicates its anticoagulant potential.

In the present study the WBC and platelet count was seen significantly increased in rats treated with L-NAME compared to group without L-NAME. WBC play an important role in thrombosis (Cheuk-kiet et al., 2003). Leukocyte accumulation and fiber deposition in thrombi mediates adhesion of monocytes and platelets leading to initiation of coagulation. Platelets play an important role in the pathogenesis of thrombi formation (Jacobi et al., 1981). Infusion of TCW reversed the levels of WBC and platelets to near normal. The antithrombotic effect of TCW may be through nitric oxide-dependent mechanism. L-Arginine administration has been reported to decrease platelet aggregation enhancing nitric oxide formation (Stief, 2001). L-Arginine through enhancement of nitric oxide synthesis in endothelium activates intraplatelet guanylate cyclase, thus increasing cyclic Guanosine Mono Phosphate (cGMP) concentration, and prevents platelet adhesion and aggregation (Bode - Boger, 1998).

The higher levels of fibrin, fibrinogen and factor V reflect hypercoagulability in fructose fed rats. Fibrinogen is a good variable that is independently related to other cardiovascular risk factors (Wohrle et al., 2003). The interaction of platelets with fibrinogen mediates a variety of responses including adhesion, platelet aggregation and fibrin clot retraction. Infusion of
TCW lowered the levels of fibrinogen and fibrin reducing the risk of clot formation. L-Arginine present in TCW may contribute to lower level of fibrinogen. The increase in fibrinogen levels is reported to be reversed by L-arginine in rats (Kawabata, 1996).

Thus the antithrombotic activity of L-arginine in TCW is a result of reduction in blood pressure associated with the suppression of platelet aggregation and increased fibrinolytic activity. It is important to note that administration of TCW without L-NAME increases urinary nitrite excretion which elevates NO leading to favourable changes in the lipid levels and coagulation factors. The administration of TCW treated with L-NAME blocks the beneficial effects due to nitric oxide synthase inhibition caused by L-NAME.

The results indicate that L-Arginine present in TCW increases NO availability and ameliorates the alterations in lipid levels and coagulation parameters in fructose induced hypertension.