INFLUENCE OF CARVEDILOL ON LIPID PROFILE AND OXIDATIVE STRESS IN HYPERLIPIDEMIC RATS

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ABSTRACT
Hypertension is a major health problem, being one of the leading cause of morbidity and mortality worldwide and a major risk factor for cardiovascular diseases. About 40% of hypertensive patients have high blood cholesterol levels and factors that increase risk for cardiovascular diseases in hypertensive individuals are increased low-density lipoprotein cholesterol (LDL), smoking, impaired glucose tolerance and reduced high density lipoprotein cholesterol (HDL) level (Lamina, 2012). Free radical production has also been reported to be increased in hypertensive patients and increased blood pressure appears to be the most important contributing factor for the generation of reactive oxygen species (Banappa, 2009). Therefore, it is important to find antihypertensive drug that improves lipid profile and also reduces oxidative stress in hypertensive patient. But among the currently available drugs, the choices are very limited.

INTRODUCTION
Hypertension is a major public health problem, being one of the leading cause of morbidity and mortality worldwide and a major risk factor for cardiovascular diseases. About 40% of hypertensive patients have high blood cholesterol levels and factors that increase risk for cardiovascular diseases in hypertensive individuals are increased low-density lipoprotein cholesterol (LDL), smoking, impaired glucose tolerance and reduced high density lipoprotein cholesterol (HDL) level (Lamina, 2012). Free radical production has also been reported to be increased in hypertensive patients and increased blood pressure appears to be the most important contributing factor for the generation of reactive oxygen species (Banappa, 2009). Therefore, it is important to find antihypertensive drug that improves lipid profile and also reduces oxidative stress in hypertensive patient. But among the currently available drugs, the choices are very limited.

Beta blockers have been used as a first line treatment of hypertension, since last four decades. Apart from anti-hypertensive action, they also have anti-anginal and anti-arrhythmic actions which effectively reduce coronary artery disease and ultimately death (Soanker, 2012). Carvedilol is a vasodilating, beta-adrenoceptor antagonist currently marketed for the treatment of hypertension. Carvedilol blocks peripheral vascular alpha 1-adrenoceptors and produces systemic arterial vasodilation to reduce total peripheral resistance while at the same time inhibits reflex tachycardia through the blockade of beta-adrenoceptors which are present in heart. Carvedilol may have other potential beneficial effects as an antioxidant and as an anti-proliferative agent (Vaidyanathan, 2009). To the best of our knowledge, very few
studies were conducted in past to evaluate effect of Carvedilol on lipid profile and oxidative stress. Hence, this study was undertaken to estimate influence of Carvedilol on lipid profile and oxidative stress in hyperlipidemic rats.

MATERIALS AND METHODS

**Animals**
Male albino rats weighing 200-250 gm were used for this experiment. They were kept on balanced diet and water *ad libitum* in a well-ventilated animal unit. Permission for conduction of study was taken from Institutional Animal Ethics Committee.

**Drugs**
Carvedilol drug was obtained as a gift sample from Dr. Reddy’s laboratories Ltd, India. Cholesterol and bile salt were purchased in pure form from Yucca Enterprises, Wadala (E) Mumbai, India. All other chemicals and reagents used in the present study were of analytical grade.

**Study Design**
Study was conducted as follows:
After 10 days adaptation period, 24 animals were divided into four groups, each containing six animals (n=6). The groups were treated as follows for four weeks: Group I: Control group (Only standard diet is given). Group II: Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt of the weight of the total diet to induce hyperlipidemia (Visavadiya, 2005). Group III: Standard diet mixed with 0.75gm% cholesterol and 1.5 gm% bile salt to induce hyperlipidemia, along with Carvedilol 10mg/kg/day orally (Rodríguez, 2001). Group IV: Standard diet mixed with 0.75gm% cholesterol and 1.5 gm% bile salt to induce hyperlipidemia, along with Carvedilol 20mg/kg/day orally (Rodríguez, 2001).

**Collection of Blood Samples**
On 30th day, after overnight fasting, blood was collected directly from heart of rat anaesthetized with ether. Abdomen was opened by taking a midline incision. Blood was sent to biochemistry laboratory; plasma was separated by centrifugation. Liver was excised and, both plasma and liver were kept frozen until analyzed.

**Biochemical Analysis**
Plasma lipid profile was assessed by following parameters by standard methods: serum total cholesterol by Modified Roeschlau’s Method (Roeschlau, 1974), serum total triglycerides (TG) by method of Wako, modified by McGowan and Fossati (McGowan, 1983), serum total high density lipoproteins (HDL) by Phosphotungstic Acid method (Klaus Loreniz, 1979), serum total low density lipoproteins (LDL) and serum total very low density lipoproteins (VLDL) by Friedewald formula (Chatterji, 2007).
Antioxidant potential was assessed by following parameters: Hepatic ascorbic acid by Schaffert RR et al method (Schaffert, 1955), catalase activity in liver by Cohen G et al method (Cohen, 1970), serum malondialdehyde (MDA) by Pasha and Sadasivadu method (Pasha, 1984), serum superoxide dismutase activity (SOD) by Marklund and Marklund method (Marklund, 1974).

**Statistical Evaluation**
The results are expressed as means ± SD (standard deviation). Significant differences among groups were determined by one way analysis of variance (ANOVA) followed by Dunnett’s test. Differences were considered significant if P < 0.05 (Mahajan, 2006).

RESULTS

**Plasma Lipid Profile**
Carvedilol 10mg/kg/day and 20mg/kg/day as drug treatments to hyperlipidemic rats resulted in no decreases in total serum cholesterol and serum LDL. The cholesterol level increased (in group IV) from 233.62±11.35mg% to 243.23 ± 10.23mg% (Table No.1). But alteration in total serum HDL was significant (P < 0.05).
Table 1: Effect of Carvedilol on serum cholesterol, LDL and HDL level in male Albino rats

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Treatment given</th>
<th>Sr. TC (mg/dl)</th>
<th>Sr. LDL (mg/dl)</th>
<th>Sr. HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>128.19 ± 6.11</td>
<td>50.94 ± 5.81</td>
<td>66.78 ± 2.24</td>
</tr>
<tr>
<td>Group II</td>
<td>HL</td>
<td>233.62 ± 11.35</td>
<td>180.45 ± 10.07</td>
<td>42.95 ± 1.94</td>
</tr>
<tr>
<td>Group III</td>
<td>HL+10CV</td>
<td>224.25 ± 10.35</td>
<td>170.13 ± 12.07</td>
<td>45.22 ± 3.01NS</td>
</tr>
<tr>
<td>Group IV</td>
<td>HL+20CV</td>
<td>243.23 ± 10.23</td>
<td>183.23 ± 12.82</td>
<td>48.56 ± 3.21*</td>
</tr>
</tbody>
</table>

(All values are Mean ±Standard Deviation). HL = Hyperlipidemic group, HL + 10CV = Hyperlipidemic group+ 10mg/kg/day Carvedilol, HL + 20CV = Hyperlipidemic group+ 20mg/kg/day Carvedilol, TC = Total Cholesterol, LDL = low density lipoproteins, HDL = high density lipoproteins, NS= Non-significant, *P < 0.05 as compared to group II (ANOVA followed by Dunnett’s test).

There were no significant decreases in serum triglyceride and serum VLDL level in Carvedilol treated groups. The values were changed from 56.98±4.08 mg% to 53.93 ± 3.87 mg% and from 11.39±0.86 mg% to 10.76 ±0.76 mg% in case of triglyceride and VLDL, respectively, in Carvedilol (20mg/kg/day) treated rats (Table No.2).

Table 2: Effect of Carvedilol on serum TG and serum VLDL level in male Albino rats

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Treatment given</th>
<th>Sr. TG (mg/dl)</th>
<th>Sr. VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>51.43 ± 2.75</td>
<td>10.36 ± 0.55</td>
</tr>
<tr>
<td>Group II</td>
<td>HL</td>
<td>56.98 ±4.08</td>
<td>11.39 ± 0.86</td>
</tr>
<tr>
<td>Group III</td>
<td>HL+10CV</td>
<td>52.84 ± 3.75NS</td>
<td>10.56 ±0.78NS</td>
</tr>
<tr>
<td>Group IV</td>
<td>HL+20CV</td>
<td>53.93 ± 3.87NS</td>
<td>10.76 ±0.76NS</td>
</tr>
</tbody>
</table>

(All values are Mean ±Standard Deviation). HL = Hyperlipidemic group, HL + 10CV = Hyperlipidemic group+ 10mg/kg/day Carvedilol, HL + 20CV = Hyperlipidemic group+ 20mg/kg/day carvedilol, TG = Total triglycerides, VLDL = very low density lipoproteins, NS= Non-significant as compared to group II (ANOVA followed by Dunnett’s test).

Antioxidant Potential

Total ascorbic acid and catalase activity in liver were increased from 44.67 ±3.61 to 50.93 ± 3.59mc/g and 13.81 ± 0.64 to 15.07 ±0.88 nm, respectively, in Carvedilol (20mg/kg/day) treated rats (Table No.3). These reductions were statistically significant (P < 0.05).

Table 3: Effect of Carvedilol on total ascorbic acid and activities of catalase in liver of male Albino rats

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Treatment given</th>
<th>Total ascorbic acid (mc/g)</th>
<th>Catalase nm H2O2 decomposed/sec/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>55.93 ± 2.85</td>
<td>21.01 ± 0.57</td>
</tr>
<tr>
<td>Group II</td>
<td>HL</td>
<td>44.67 ±3.61</td>
<td>13.81 ± 0.64</td>
</tr>
<tr>
<td>Group III</td>
<td>HL+10CV</td>
<td>48.83 ± 3.59NS</td>
<td>14.17 ±0.88*</td>
</tr>
<tr>
<td>Group IV</td>
<td>HL+20CV</td>
<td>50.93 ± 3.59*</td>
<td>15.07 ±0.87*</td>
</tr>
</tbody>
</table>

(All values are Mean ±Standard Deviation). HL = Hyperlipidemic group, HL + 10CV = Hyperlipidemic group+ 10mg/kg/day Carvedilol, HL + 20CV = Hyperlipidemic group+ 20mg/kg/day carvedilol, NS= Non-significant, *P < 0.05 as compared to group II (ANOVA followed by Dunnett’s test).
The lipid peroxidation product, malondialdehyde, in serum decreased in 20mg/kg/day Carvedilol treated rats as compared to hyperlipidemic group i.e. from 3.57±0.43 nmol/ml to 3.13±0.41 nmol/ml (Table No. 4). But this reduction was not statistically significant (P = 0.15).

### Table 4: Effect of Carvedilol on serum MDA and serum SOD level in male Albino rats

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Treatment given</th>
<th>Sr. MDA (nmol/ml)</th>
<th>Sr. SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>1.44 ± 0.28</td>
<td>11.99 ± 0.54</td>
</tr>
<tr>
<td>Group II</td>
<td>HL</td>
<td>3.57 ±0.43</td>
<td>5.98 ± 0.83</td>
</tr>
<tr>
<td>Group III</td>
<td>HL+10CV</td>
<td>3.43 ± 0.44&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>7.25 ± 0.79*</td>
</tr>
<tr>
<td>Group IV</td>
<td>HL+20CV</td>
<td>3.13 ± 0.41&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>7.35 ± 0.87*</td>
</tr>
</tbody>
</table>

(All values are Mean ±Standard Deviation). HL = Hyperlipidemic group, HL + 10CV = Hyperlipidemic group+ 10mg/kg/day Carvedilol, HL + 20CV = Hyperlipidemic group+ 20mg/kg/day carvedilol, MDA = Malondialdehyde, SOD = Superoxide dismutase. NS = Non significant,* P < 0.05 as compared to group II ((ANOVA followed by Dunnett’s test).

The activity of superoxide dismutase enzyme increased in Carvedilol treated rats (group IV) as compared to hyperlipidemic group i.e. from 5.98±0.83 U/ml to 7.35 ± 0.87 U/ml (Table No. 4). This increase in superoxide dismutase activity was statistically significant (P < 0.05).

### DISCUSSION

Oxidative stress has emerged as an important pathogenic factor in the development of hypertension and also most of the complications related to hypertension are associated with oxidative stress, induced by the generation of free radicals (Soanker, 2012). Therefore, treatment compounds having both lipid lowering and antioxidant properties would be useful as anti-hypertensive agents. Hence, the present study was conducted to estimate influence of Carvedilol, a third generation beta blocker, on lipid profile and oxidative stress in hyperlipidemic rats. The lipid-lowering effects of Carvedilol in hyperlipidemic rats, demonstrated in the present investigation, were related primarily to increase in HDL-cholesterol level only and no improvement in levels of total serum cholesterol, LDL-cholesterol, triglycerides and VLDL-cholesterol.

In past, one study was conducted to compare the effects of Carvedilol and captopril on serum lipid concentrations in patients with mild to moderate essential hypertension and dyslipidaemia. In this study, Carvedilol improved all the parameters of lipid profile (Hauf-Zachariou, 1993). Sharp RP et al, in their study, studied the impact of carvedilol on the serum lipid profile and concluded that carvedilol had a potentially negative effect on high-density lipoprotein cholesterol (Sharp, 2008). Carvedilol is a vasodilating, beta-adrenoceptor antagonist currently marketed for the treatment of mild to moderate hypertension. This drug having alpha and beta blocker properties which are responsible for decrease and increase in lipid levels, respectively (Vaidyanathan, 2009).

Elevated levels of lipids are associated with atherosclerosis and predispose to cardiovascular disease (Durrington, 2003). High level of HDL-C is associated with fewer problems with cardiovascular diseases and vice versa. It is very clear that an increase in HDL-C level could potentially contribute to reversal of process of atherosclerosis. This is because high level of HDL-C protects endothelial cells from the cytotoxic effects of oxidized LDL (Assmann, 2003). In the present study, a significant increase in plasma HDL-C level definitely indicates the beneficial role of Carvedilol administration to hyperlipidemic animals.

The importance of the reactive oxygen species has attracted increasing attention over the last decade. They are involved in pathogenesis of various serious diseases such as neurodegenerative disorders, cancer, cardiovascular diseases and inflammation. Thus, oxidative stress is a cardinal in the pathogenesis of hypertension and atherosclerosis. Understanding the mechanisms of oxidative stress and the means of
suppressing it are important in controlling complications related to atherogenesis. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing complications of such type of oxidative stress related diseases (Singh, 2008).

Presently noted increased levels of catalase and superoxide dismutase enzyme activities and also increase in ascorbic acid level in Carvedilol treated group indicate the possible role of Carvedilol as an antioxidant. In past, many studies were conducted with Carvedilol to confirm its antioxidant activity (Noguchi, 2000 and Dandona, 2007). The antioxidant activity of carvedilol could be explained by a greater degree of lipophilicity and also the molecular structure of carvedilol favors redox recycling. Therefore, carvedilol could have additional pharmacologic effects that are favorable for long-term therapy (Lysko, 2000). Taken together, these observations indicate that Carvedilol administration to hyperlipidemic animals can increase HDL-C level and improve antioxidant enzyme activities.

Thus, we conclude that Carvedilol could increase serum HDL-C as well as decrease oxidative stress in hyperlipidemic conditions which suggest that Carvedilol may reduce certain complications in patients of hypertension by its lipid lowering and antioxidant potential.

REFERENCES
Research Article


