Nitric Oxide and Oxidative Stress Properties of L-Carnitine in Diabetic Hypertensive Rats Biochemical & Histological Study

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ABSTRACT

Background: Diabetes mellitus and hypertension are a global health problem due to their serious complications, which along with oxidative stress have been shown to contribute to endothelial dysfunction.

Aim: The aim of the present study was to investigate the role of the beneficial effect of L-carnitine in healthy and streptozotocin (STZ) NO-nitro-L-arginine methyl ester (L-NAME) induced diabetes mellitus and hypertension in rats.

Results: Results showed that diabetic hypertensive (DH) rats had significant increase in the level of plasma glucose, malondialdehyde (MAD), cholesterol (CH), triglycerides (TG), urea, creatinine, and the activity of serum liver enzymes (AST, ALT) compared to normal control rats. Blood glutathione (GSH) content and erythrocyte superoxide dismutase (SOD) activity and nitric oxide level (NO) were significantly lowered. Supplementation of L-carnitine for 6 weeks improved plasma glucose, lipids, liver and kidney functions. In addition, both normal healthy rats and DH rats treated with L-carnitine showed increase in blood GSH and SOD activity and serum nitrate level (stable product of nitric oxide NO) as compared with healthy controls and DH respectively. Histopathological and immunochemical study of heart confirmed the biochemical results.

Conclusion: It was concluded that administration of L-carnitine reduces or delays oxidative stress in diabetic hypertensive rats.

Key words: L-carnitine - L-NAME- Nitric Oxide, Oxidative Stress, Diabetic rats, Hypertensive rats, Histological Study.
Introduction
Diabetes results in a state of increased reactive oxygen species (ROS) production, and oxidative stress is implicated in the development and progression of various diabetic complications (Baynes, 1991; Yao et al. 2009). Increased oxidative stress is thought to play an important role in the etiology and pathogenesis of chronic complications of diabetes (Scott and King, 2004; Yao et al., 2009).

The antidiabetic actions of individual free amino acids are of great interest. Intervention of glycation may prove to be beneficial to patients suffering from diabetes mellitus. Free amino acids are known to mitigate the glycation of lens protein, delay cataractogenesis and bring down blood sugar levels in diabetic rats and promote tissue sensitivity towards insulin. Further, amino acids inhibit the binding of glucose with proteins, the first step in the pathway of glycation cascade by competitive inhibition, thereby offering protection (Anuradha, 2009).

Arterial hypertension is associated with a high production of reactive oxygen species and a decrease in the antioxidant defence systems. Since oxidative stress has gained importance in the last few years as one of the mechanisms involved in the origin and development of hypertension, and considering that L-carnitine (LC) is a useful compound in different pathologies characterized by increased oxidative status, the aim of this work was to test the hypothesis that LC might protect the heart against hypertension-induced oxidative damage. In spite of a wide range of drugs being available in the market, treatment of arterial hypertension still remains a challenge, and new therapeutic strategies could be developed in order to improve the rate of success in controlling this disease (Zambrano et al., 2013).

The importance of L-carnitine (b-hydroxy-y-N-trimethylammonium butyric acid) for the lipid and carbohydrate metabolism has been long established. Carnitine is required to transport long-chain fatty acids from the cytoplasm to the mitochondrial matrix where their oxidation occurs, and on the other hand, carnitine increases the sensitivity of the cells to insulin and the use of glucose by the peripheral tissues (Mamoulakis et al., 2004).

Some other effects of carnitine on cellular metabolism as protection against oxygen free radicals and of mitochondrial biogenesis in aged rats were demonstrated. Carnitine influence membrane fluidity, ion channel function, and smooth muscle contractility. It was suggested that membrane effects are implicated in the mechanism by which carnitine derivatives protect the heart from ischemia or oxidative stress. This might be in concert with findings on the changes of cardiac carnitine metabolism in various hypertensive models (Rauchova, 1998).

Nitric oxide (NO) is the most pivotal molecule secreted by endothelium and thus is a major mediator of endothelial function. The production of NO is catalyzed by a family of enzymes called nitric oxide synthases (NOS), which convert the amino acid L-arginine to L-citrulline and NO, apart from playing an important role in vasodilation. Evidence suggests that NO plays a major role in regulating blood pressure and glucose levels, and thus impaired NO bioactivity forms an important component of hypertension and diabetes. The physiological importance of NO in the regulation of blood pressure is evidenced by the fact that pharmacological inhibition of NO synthases leads to severe hypertension, vascular injury, and glomerulosclerosis in experimental animals (Shiekh et al., 2011).

Material and Methods
Experimental design and animal grouping
Design: 40 white male albino rats weighing 150-200 g. were used for this study. All animals were housed in stainless steel cages, 10 per cage under controlled environmental conditions. Diabetes was induced in male Wister albino rats by single intraperitoneal injection of 50 mg/kg streptozotocin (STZ) (Heo et al., 2002). Four days after STZ injection, rats received N-O-nitro-L-arginine methylester (L-NAME) (0.5 mg/mL in drinking water for 4 weeks) for induction of hypertension (Zambrano et al., 2013). L-carnitine (0.5 g/100 gm diet) was given daily to DH rats for six weeks, respectively (Oka et al., 2008).

Our work was carried out in accordance with the guidelines of Faculty of Applied Medical Science at Taif University in K.S.A. for animal use.

Handling of the animal was the same for all groups and did not affect weight gain.

Groups:
1- Group 1: Normal control: 10 rats fed the balanced diet during the entire study (10 weeks).
2- Group 2: L-carnitine: 10 rats fed the balanced diet supplemented with L-carnitine during the entire study (10 weeks).
3- Group 3: Diabetic hypertensive group (DH): this group contains 10 diabetic hypertensive rats.
4- Group 4: Diabetic hypertensive group treated with L-carnitine (DH+L-car): 10 diabetic hypertensive rats receive L-carnitine as treatment for 6 weeks.

Our goal is to achieve a diabetic hypertensive model in 4 weeks following treatment period for 6 weeks. This model provided us with a reliable method and resembles the clinical cases of diabetic hypertensive rats and their treatment. After six weeks treatment, heart rate and blood pressure of all animals were recorded by the tail cuff method using the Harvard Blood Pressure Monitor. Blood samples were collected from the tail vein of lightly anesthetized overnight fasted animals from all the groups.

Sample collection and analytical methods
By the end of the experimental periods, blood samples were collected in two centrifuge tubes. One tube contained
heparinized blood used for determination of SOD and GSH activity. In the second tube serum was separated by leaving blood sample for 15 minutes at a temperature of 25 °C then it was centrifuged for 20 minutes at 4000 r.p.m., using clean dry disposable plastic syringes and stored at -20°C for subsequent biochemical measurements as follows: lipid profile (TC, TG) (Fossati and Prencipe, 1982), liver enzyme activities related to its function (ALT, AST) (Reitman and Frankel, 1957), kidney function (urea and creatinine) (Li, 1996), heart biomarkers (LDH, CK) (Würzburg et al., 1977), glucose (Trinder, 1969).

Lipid peroxidation and oxidative stress were measured through malondialdehyde (MDA), nitric oxide (NO), oxidative stress markers, reduced glutathione (GSH) and superoxide dismutase activity (SOD) according to Mahesh et al. (2009).

Histological Examination: Heart specimens from each group were examined histopathologically. Heart tissues were fixed in 10% formalin solution for 48 hours and embedded in paraffin wax, sectioned (4 um), and then stained with hematoxylin and eosin. Pathological changes were evaluated in the tissues as previously described (Helin HO 2006), De Rossi A 2007).

Immunohistochemical staining:

Immunohistochemistry was conducted on paraffin embedded heart sacrifices from control and treated rats. Slides were deparaffinized, dehydrated, washed in phosphate buffer saline then covered with peroxide block staining (PBS) to block off endogenous staining and incubated for 10 minutes at room temperature in a humidity chamber. Monoclonal mouse caspase-3 antibody (Lab vision, USA) (Cai, L 2002) was applied on the tissue sections then incubated horizontally in a humidity chamber for an hour, at room temperature. After removal of excess buffer, the sections were incubated in pre-formed strept avidin peroxidase. DAB substrate- chromogen (3,3- Diaminobenzidine tetrahydrochloride) was applied on slides for 5-15 minutes until the desired brown color was obtained. Counter - staining was done by using Mayer’s haematoxylin.

Image Analysis

The data were obtained using Lecia Qwin 500 image analyzer computer system (England). The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the microscope, and controlled by Lecia Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The area and area % of caspase-3 immunoexpression in myocardial sections of the animals of all groups were measured using an objective lens of magnification 10, i.e. of total magnification of 100. Ten random fields were measured for each specimen. In each chosen field, the areas of caspase-3 immunoactivity were enclosed inside a standard measuring frame (Figure 1 - below).

Statistical Analysis

Statistical analysis was carried out using Graph Pad Instat software (version 3, ISS-Rome, Italy). Unless otherwise specified, groups of data were compared with an unpaired t-test one-way analysis of variance (ANOVA). Values of P < 0.05 were regarded as significant. Data were expressed in tables as mean ± standard error (SE) (Altman and Bland 1996).
Biochemical Results
Effect of L-carnitine on hypertension, heart rates and heart weight:

The current results tabulated in Table 1 administration of L-carnitine by healthy and DH rats showed that there is a significant decrease in hypertension in the diabetic hypertensive L-carnitine treated group (STZ+L-NAME +L-car) compared to the untreated group (STZ+L-NAME), heart rates also showed that there is a significant amelioration after treatment with L-carnitine (P < 0.05).

Table 1: Effect of L-carnitine (L-car) on the blood pressure, heart rates and mean heart weight in control and diabetic hypertensive (STZ+L-NAME) rats

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean blood pressure (mmHg)</th>
<th>Heart rate (beats/minutes)</th>
<th>Mean heart weight (Gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107.0 ± 5.0</td>
<td>314 ± 20</td>
<td>0.89 ± 0.09</td>
</tr>
<tr>
<td>L-car</td>
<td>90.0 ± 3.0</td>
<td>331 ± 25</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>STZ+L-NAME</td>
<td>139.0 ± 5.0</td>
<td>270 ± 24</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td>STZ+L-NAME +L-car</td>
<td>118.0 ± 4.0</td>
<td>293 ± 27</td>
<td>0.92 ± 0.04</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, n= 10 a and b indicate significant change from control, STZ+L-NAME, respectively at p≤ 0.05 using ANOVA test.

Diabetic hypertensive rats (STZ+L-NAME), showed decreased heart weights that was statistically significant as compared with controls (group I). On the other hand, in animals of (STZ+L-NAME +L-car), the loss of heart weights was statistically insignificant as compared with controls (Table 1).

Effect of L-carnitine on blood glucose and lipid profile:

Results tabulated in Table 2 revealed that administration of L-carnitine at tested doses by healthy rats showed a non significant decrease in serum glucose level. While administration of L-carnitine at the same doses by DH rats showed a significant decrease in glucose level p< 0.05. Regarding serum CH, TG and LDL-C levels our results showed a significant decrease in the DH+L-carnitine group than the diabetic hypertensive group, while HDL-C showed a significant increase P ≤ 0.05.

Table 2: Effect of L-carnitine (L-car) on the levels of blood glucose, triglycerides, total cholesterol, high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) in control normal and diabetic hypertensive (STZ+L-NAME) rat serum

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Glucose (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.3±0.5</td>
<td>79.7±0.5</td>
<td>82.3±0.9</td>
<td>40.6±0.4</td>
<td>38.6±0.8</td>
</tr>
<tr>
<td>L-car</td>
<td>86.9±0.5</td>
<td>77.8±0.7</td>
<td>83.5±1.7</td>
<td>42.1±0.6</td>
<td>37.9±0.9</td>
</tr>
<tr>
<td>STZ+L-NAME</td>
<td>215.0±1.0</td>
<td>126.9±0.9</td>
<td>150.2±1.8</td>
<td>18.6±0.5</td>
<td>101.1±1.9</td>
</tr>
<tr>
<td>STZ+L-NAME +L-car</td>
<td>150.5±0.8</td>
<td>84.3±0.5</td>
<td>93.3±1.4</td>
<td>32.7±1.1</td>
<td>64.2±1.9</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, n= 10 a and b indicate significant change from control, STZ+L-NAME, respectively at p ≤ 0.05 using ANOVA test.
Effect of L-carnitine on ALT, AST, LDH, CK, urea and creatinine, in healthy and the corresponding diabetic hypertensive rats:

The results tabulated in Table 3 illustrate the effect of L-carnitine on serum ALT, AST, LDH, CK, urea and creatinine, in healthy and diabetic hypertensive rats. The results revealed that L-carnitine supplementation to the healthy rats resulted in a non significant change compared to untreated healthy rats. While administration of L-carnitine by DH rats showed a significant decrease ($P < 0.05$) in the levels of ALT, AST, LDH, CK, urea and creatinine as compared with the diabetic hypertensive control group.

Table 3: Effect of L-carnitine (L-car) on the levels of Liver enzyme activities (ALT, AST), cardiac enzyme activities (LDH, CK) and kidney functions (urea, creatinine), in control normal and diabetic hypertensive (STZ+L-NAME) rat serum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (u/l)</th>
<th>AST (u/l)</th>
<th>LDH (u/l)</th>
<th>C.K-NAC (u/l)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.1 ± 0.9</td>
<td>24.9 ± 0.9</td>
<td>258.5 ± 10.8</td>
<td>250.2 ± 12.6</td>
<td>28.6 ± 0.9</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>L-car</td>
<td>15.3 ± 0.9</td>
<td>22.8 ± 1.1</td>
<td>241.7 ± 10.6</td>
<td>242.2 ± 10.6</td>
<td>26.5 ± 1.3</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>STZ+L-NAME</td>
<td>24.6 ± 0.6</td>
<td>41.8 ± 11.1</td>
<td>472.8 ± 6.4</td>
<td>407.4 ± 8.9</td>
<td>52.8 ± 0.71</td>
<td>1.6 ± 0.04</td>
</tr>
<tr>
<td>L-car+STZ+L-NAME</td>
<td>20.6 ± 0.9</td>
<td>34.2 ± 1.1</td>
<td>397.9 ± 8.2</td>
<td>340.4 ± 8.9</td>
<td>37.3 ± 0.71</td>
<td>0.82 ± 0.03</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, n= 10 a and b indicate significant change from control, STZ+L-NAME, respectively at $p< 0.05$ using ANOVA test.

Effect of L-Carnitine on GSH, SOD, NO and MDA in healthy and their corresponding diabetic hypertensive rats:

Regarding thblood (GSH) and erythrocyte (SOD) activity, serum NO results tabulated in Table 4 revealed that consuming L-carnitine either by healthy and diabetic hypertensive groups showed a significant increase in these values as compared with their corresponding groups. Our results showed also administration of L-carnitine by the healthy rats (group 2) (L-car) caused a non significant reduction in the serum level of MDA as compared with control (group one). On the other hand, administration of L-carnitine by DH rats resulted in a significant reduction ($P < 0.05$) on serum MDA compared with diabetic hypertensive untreated rats.

Table 4: Effect of L-Carnitine on Glutathion reductase (GSH), superoxide dismutase (SOD), nitric oxide (NO), and malondialdehyde (MDA) in control normal and diabetic hypertensive (STZ+L-NAME) rat (mean ±SE).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (mg/gHb)</th>
<th>SOD (units/ml)</th>
<th>NO (µmol/L)</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68.7 ± 0.8</td>
<td>334.7 ± 25.8</td>
<td>98.7 ± 12.2</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>L-car</td>
<td>72.6 ± 0.7</td>
<td>360.4 ± 29.3</td>
<td>102.3 ± 14.2</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>STZ+L-NAME</td>
<td>37.3 ± 0.7</td>
<td>197.12 ± 41.3</td>
<td>40.7 ± 12.3</td>
<td>9.9 ± 0.3</td>
</tr>
<tr>
<td>L-car+STZ+L-NAME</td>
<td>54.4 ± 0.7</td>
<td>303.7 ± 34.5</td>
<td>78.4 ± 22.3</td>
<td>5.2 ± 0.12</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, n= 10 a and b indicate significant change from control, STZ+L-NAME, respectively at $p< 0.05$ using ANOVA test.

Effect of L-Carnitine on mean area % of caspase-3 immunoreactivity in the myocardium in control normal and diabetic hypertensive rats: Table 5 shows mean area % of caspase-3 immunoreactivity in the myocardium of control and treated groups:
Table 5: Effect of L-Carnitine on mean area % of caspase-3 immunoreactivity in the myocardium in control normal and diabetic hypertensive rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean area % of caspase-3 immunoreactivity in the myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.04 ± 1.9</td>
</tr>
<tr>
<td>L-car*</td>
<td>6.08 ± 2.01</td>
</tr>
<tr>
<td>STZ+L-NAME*</td>
<td>26.33 ± 15.17</td>
</tr>
<tr>
<td>STZ+L-NAME+L-car*↑</td>
<td>8.79 ± 4.86</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE, n= 10 a and b indicate significant change from control, STZ+L-NAME, respectively at p≤ 0.05 using ANOVA test.

Histological Results
Diabetic hypertensive treated animals of (group III), showed increased heart weights that was statistically highly significant as compared with controls (group I). On the other hand, in animals of (group IV), the loss of heart weights was statistically insignificant as compared with controls (Table 1). Mean area % of caspase-3 immunoreactivity in the myocardium of control and treated groups shown in Table 5.

Light microscopic results of control heart sections (group I) stained with H&E, the myocardium showed branching and anastomosing muscle fibers with centrally located oval basophilic euchromatic nuclei and connected together by intercalated discs (Figure 2). In L-carnitine treated control animals (group II), the myocardium appeared nearly with normal structure as control rats (fig.3). Infiltrating inflammatory cells were observed in the left ventricle sections of D H rats, and these cells were usually found in clusters of cells located throughout the interstitium with congested blood vessels (Figure 4). Treatment with L-carnitine in (group IV) showed markedly reduced inflammatory cell infiltration in diabetic hypertensive rats however, some inflammatory cells were still found within the interstitial component of the left ventricle (Figure 5).

Figure 2: A photomicrograph of a longitudinal section of the myocardium of a control adult rat (group I) showing branching muscle fibers with centrally located oval nuclei (arrows). Note: intercalated discs (lines) and flat dark nuclei of the fibroblasts (white arrow) of the connective tissue endomysium. (Hx. & E. X 400)
Figure 3: A photomicrograph of a longitudinal section in the myocardium of an adult rat from group II (L-carnitine control rats), showing normal myocardium which appeared nearly the same as control rats. (Hx. & E. X 400)

Figure 4: A photomicrograph of a longitudinal section in the myocardium of an adult rat from group III (diabetic hypertensive rats), showing inflammatory cell infiltration (arrow) & congestion of blood vessels (2 heads arrow) with some degeneration in myocardium rats (white arrow). (Hx. & E. X 400)
Figure 5: A photomicrograph of a longitudinal section in the myocardium of an adult rat from group IV (l-carnitine treated diabetic hypertensive rats), showing myocardium with some inflammatory cells (arrows). (Hx. & E. X 400)

Immunohistochemical and image analysis results:
Myocardial sections of control animals (group I) showed a negative immune reaction to caspase - 3 (Figure 6) i.e. no brownish color in the cytoplasm (apoptosis). Group II (LC treated animals) like controls showed a negative immune reaction to caspase - 3 (Figure 7).

Figure 6: A photomicrograph of a longitudinal section of the myocardium of a control adult rat (group I) showing negative immune reaction to caspase - 3. (Caspase-3 Immunoreactivity, X 400)
Figure 7: A photomicrograph of a longitudinal section of the myocardium of an adult rat from group II, (l carnitine treated animals) showing negative immune reaction to caspase-3 (arrows). (Caspase-3 Immunoreactivity, X 400).

Diabetic hypertensive treated animals (group III), showed increased area of positive immune reaction to caspase−3 (Figure 8). In group IV, there were few areas with positive immune reaction to caspase - 3 (Figure 9). Diabetic hypertensive treated animals (group III) showed a highly significant increase in the mean area % of caspase-3 immunoreactivity in the myocardium as compared with controls. However the (Group VI) showed a statistically significant decrease in the mean area % of caspase-3 expressions (Table 5).

Figure 8: A photomicrograph of a longitudinal section of the myocardium of an adult rat from group III, (diabetic hypertensive animals) showing very strong positive immune reaction to caspase−3 (arrows). (Caspase-3 Immunoreactivity, X 400)
Discussion

Reduced NO availability in diabetes mellitus and hypertension, underlines its relevance to the development of secondary complications in these clinical conditions. Alteration of NO metabolism and increased oxidant stress, was demonstrated to be involved in the pathogenesis of macrovascular events, which are increased in hypertensives as well as diabetics. Results of the present study revealed that administration of L-carnitine by healthy and diabetic hypertensive rats caused a significant decrease in hypertension, while, resting heart rates were increased as compared to their corresponding controls. These results are in agreement with Ruggenenti et al (2009) and Malton et al (2006). They suggest, that a deficiency of free carnitine can manifest as elevated free fatty acids, suggesting a reduction of inter-cellular transport. Elevating free carnitine levels in diabetic animals with a fixed and relatively inadequate availability of glucose as a myocardial fuel apparently corrected the defects in myocardial function by providing more intracellular fatty acids as an energy substrate.

In the present study diabetic untreated rats showed a significant elevation of serum glucose level. Our results were confirmed by (Malton et al., 2006) who illustrated that the blood glucose level of diabetic rats increased compared to control animals. Administration of L-carnitine at the tested doses by diabetic rats only showed a significant decrease in serum glucose level compared to the untreated group. These results are in agreement with Mamoulakis et al (2004) who reported that carnitine increases the sensitivity of the cells to insulin and the use of glucose by the peripheral tissues. Carnitine administration improved whole body insulin sensitivity, glucose tolerance and prevents oxidative stress.

L-carnitine supplementation produced significant decreases in serum TG, and T-cholesterol, LDL-C while there was a significant increase in HDL-C in diabetic hypertensive rats. These results are in agreement with those of González-Ortiz et al (2008) and El-Metwally et al (2003), who reported that oral L-carnitine increases plasma free carnitine levels, improves dyslipidemia and decreases oxidative stress, with reduction of cardiac parameters. L-carnitine administration to diabetic hypertensive rats reduces significantly hypertriglyceridemia (Table 2) via decreased synthesis of triglycerides by the liver or by inhibition of triglyceride release from the liver.

L-carnitine suppressed hydroxyl radical production in the Fenton system, probably by chelating their iron, is required for the generation of hydroxyl radicals. Thus, the reduction in lipid peroxidation in the present study might be due to the iron-chelating property of L-carnitine. This hypothesis is consistent with the previous study which has demonstrated that L-carnitine showed a strong antioxidant activity against irradiation-induced lipid peroxidation and has free radical scavenging effects (Mansour, 2013).

In the present work diabetic hypertensive animals showed a significant increase in the AST, ALT, CK and LDH activities (P < 0.05), also serum urea and creatinine levels. This may be due to increase in oxidative damage and decrease in antioxidant capacity of the liver which suggests that oxidative stress has an impact on liver disorders. These results are in harmony with that of Khalil (2009).

The oral administration of L-carnitine (Table 3) shows that serum concentration of urea and creatinine were significantly decreased. The effect of L-carnitine on renal lipid metabolism...
could serve as a new therapeutic approach, as it counters the renal changes associated with metabolic syndrome. Hence, L-carnitine has beneficial effects on renal function.

Anuradha (2009) reported that L-carnitine protects against liver, kidney and heart disease. L-carnitine improves heart function in diabetics and hypertensives and increases the level of glucose oxidation, a process that helps cells make use of glucose. McMackin (2007) and Ruggenenti, et.al. (2009) reported that Acetyl-l-carnitine safely ameliorated arterial hypertension, insulin resistance, impaired glucose tolerance, and hypoadiponectinemia in subjects at increased cardiovascular risk. Whether these effects may translate into long-term cardioprotection is worth investigating.

In the present work, the increased serum MDA value in diabetic hypertensive rats may be attributed to the increased level of oxygen free radicals which could be due to their increased production and/or decreased destruction by non-enzymatic and enzymatic antioxidants. Our results are in agreement with Sailaja-Devi and Das (2005), who reported a significant increase in the plasma level of MDA, as well as a significant decrease in serum level of NO(x). GSH and the activity of SOD in RBCs lyase in diabetic animals compared to healthy controls. Also Barakat (2006) illustrated that the decrease in GSH levels during diabetes is probably due to its increased utilization by the hepatic cells. This may be due to an attempt by the hepatocytes to counteract the increased formation of lipid peroxides. Tas et.al. (2007) illustrate both plasma and tissue MDA levels were significantly reduced in the diabetic group treated with individual free amino acids compared to those of the diabetic untreated group. These alternations might be related to hypoplipidemic, hypoglycemic and direct oxidative effects of free amino acids. The antioxidative and hypoglycemic effect of L-carnitine might also be involved in the changes in antioxidative enzyme activities. Our results showed that erythrocyte SOD activity was significantly increased in the diabetic hypertensive control group and the diabetic hypertensive group treated with L-carnitine compared to control group; the elevation might be due to the protective mechanism against oxidative stress.

L-carnitine effectively protects and improves mitochondrial function in vivo: it acts as an antioxidant, so by inhibiting ROS and RNS it protects the vascular endothelial tissues against oxidative damage in hypertension Gómez-Amores et al (2007). Thus, L-carnitine treatment effectively protected the liver tissue against oxidative damage and showed marked improvement in its antioxidant status.

Nitric oxide level showed a significantly reduced level (P < 0.5) in diabetic hypertensive rats (Table 4) than control ones. Various studies have reported a significant decrease of plasma nitric oxides in diabetes mellitus and hypertension; our results coincide with these reports. Shiekh et.al. (2011), presumed that the cascade of NO bioactivity and availability on smooth muscle cells was impaired in the early affected stage of diabetes mellitus and followed the decrease of endothelial NO production.

In the present study, the decreased serum NO value in diabetic hypertensive rats was ameliorated by administration of L-carnitine. Shiekh et.al.(2011), reported that Nitric oxide (NO) turnover is vital for proper endothelial function to maintain a healthy vascular system. Various risk factors responsible for hypertension and diabetes may disrupt this homeostasis, leading to decreased bioavailability and/or bioactivity of NO, which potentiates endothelial dysfunction. Plasma NO is a useful indicator of NO homeostasis and vascular endothelial function which plays a key role in the development and progression of diseases like diabetes and hypertension.

Cardiovascular remodeling includes hypertension, endothelial damage, cardiac hypertrophy, inflammation, ventricular contractile dysfunction and fibrosis (Weber KT et al 2001). L-Carnitine plays a major role, as a cofactor, in the transportation of free fatty acids from the cytosol to the mitochondria for adenosine triphosphate synthesis. An altered metabolic substrate used in the failing heart also contributes to the dysfunction of the mitochondrial electron transport chain, resulting in enhanced production of superoxide (Rosca MGetal 2008). Mitochondrial dysfunction and increased mitochondrial superoxide production, preceding endothelial dysfunction, might favour the development of hypertension (Puddu P et. al. 2008).

Free radicals also potentiate mitochondrial dysfunction by further damaging mitochondrial DNA, with resultant impairment in the synthesis of some components of the respiratory chain and further increases in superoxide production (Puddu P et. al. 2008, Esposito LA et. al. 1999 Shibutani S 1991, Zorov DB 2006). The current experiment was designed to estimate the effect of L-carnitine on nitric oxide and oxidative stress in normal and in diabetic hypertensive rats. In this study Infiltrating inflammatory cells were observed in the left ventricle sections of D H rats, and these cells were usually found in clusters of cells located throughout the interstitium with congested blood vessels. Treatment with L-carnitine in (group IV) showed markedly reduced inflammatory cell infiltration in diabetic hypertensive rats, however, some inflammatory cells were still found within the interstitial component of the left ventricle. The results of the current investigation were strongly supported by (Ferrari R, 2003, Malone JI,2003) who found that L-Carnitine treatment improved heart function after ischaemia and reperfusion injury, and also improved heart rate regulation and ventricular size in streptozotoc-indiabetic rats. In addition, the anti-hypertensive effects of L-carnitine in this study may result from inhibition of inflammation, as inflammation is an integral part of the cardiovascular remodeling observed in L-NAME hypertensive rats. Furthermore, plasma concentrations and cardiac expression of inflammatory markers such as IL-6 and TNFa were reduced after L-carnitine treatment in L-NAME.
in enhancing fatty acid oxidation (El-Beshlawy A 2008) and the consequent role to reduce the production of superoxide (G_IÅin I 2006), and further increasing the availability of nitric oxide (Rajasekar P 2007). L-carnitine controls oxidative stress by improving mitochondrial function (G_IÅin I 2006), (Calvani M, 2000). Diabetic hypertensive treated animals (group III) showed a highly significant increase in the mean area % of caspase-3 immunoreactivity in the myocardium as compared with controls. However the (Group VI) showed decrease in the mean area % of caspase-3 expressions in the present study. These results were in agreement with (Abdel Baky N, 2011), who found that l-carnitine significantly reduced the level of heart-type fatty acid binding protein, capase-3 activity, as well as myocardial DNA damage in diabetic hypertensive rats.

References


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