Cardiac apoptosis as a possible cause of diabetic cardiomyopathy and the protective role of alpha lipoic acid and Losartan in diabetic rats

Abeer A. Shoman and Noha I. Hussien
Department of physiology; Faculty of medicine; Benha University

Abstract

Background: Diabetic cardiomyopathy is a common complication of diabetes mellitus. Cell death such as apoptosis plays a critical role in cardiac pathogenesis.

AIM: This study was designed to assess the issue of cardiomyocyte apoptosis as a possible cause of diabetic cardiomyopathy and whether it would be possible to suppress this apoptosis by the use of alpha lipoic acid (ALA) as an antioxidant and Losartan as an Angiotensin II receptor blocker.

METHODS: Rats were randomly divided into five groups 10 animals each: Group 1, healthy control rats; Group 2, diabetic group that were made diabetic with a single injection of streptozotocin (STZ); Group 3, diabetic rats treated with Losartan; Group 4, diabetic rats treated with ALA; Group 5, diabetic rats treated with Losartan and ALA. At the end of the experimental period, plasma glucose was measured. The heart rate and mean systemic arterial blood pressure (MSBP) were measured in all groups. Oxidative stress as assessed by malondialdehyde (MDA) and reduced glutathione (GSH-PX) concentrations as well as caspase-3 activity as an index of apoptosis were determined in cardiac tissue. In addition Cardiac apoptosis was measured by BCL-x immunohistochemistry technique.

RESULTS: Administration of ALA and Losartan caused significant decreased in apoptosis, in addition there was significant decrease in MDA, caspase-3 and increase in GSH in cardiac tissue homogenate, and with significant decrease in the serum lipid level and mean systemic arterial blood pressure were significantly decreased while heart rate increased to normal level.

CONCLUSION: alpha lipoic acid and Losartan have a protective effect on diabetic induced cardiomyopathy in rats.

Abbreviations: Glutathione (GSH-PX) - Streptozotocin (STZ) - Mean systemic arterial blood pressure (MSBP) - Malondialdehyde (MDA) - ALA (alpha lipoic acid).
Diabetes represents a serious risk factor for the development of cardiovascular complications such as coronary heart disease, peripheral arterial disease, hypertension, stroke, cardiomyopathy. Moreover, mortality from cardiac diseases is approximately two- to fourfold higher in patients with diabetes than in those who have the same magnitude of vascular diseases without diabetes, and diabetic cardiomyopathy can occur without any vascular pathogenesis.

Diabetic cardiomyopathy is related directly to hyperglycemia. Cell death such as apoptosis plays a critical role in cardiac pathogenesis, as hyperglycemia induces myocardial apoptosis. In diabetes the circulating free radicals may contribute to progression of heart disease and possibly mediate the process of apoptosis. Hyperglycemia-induced myocardial apoptosis is mediated, at least in part, by activation of the cytochrome c-activated caspase-3 pathway, which may be triggered by ROS (reactive oxygen species) derived from high levels of glucose.

Cell death, as a consequence of myocardial abnormalities in diabetes, is an important cause of various cardiomyopathies. In particular, cell death can cause a loss of contractile tissue, compensatory hypertrophy of myocardial cells, and reparative fibrosis. Studies showed that the incidence of apoptosis increases in the heart of patients with diabetes.

Having diabetes makes high blood pressure and other heart and circulation problems more likely because diabetes damages arteries and makes them targets for hardening (atherosclerosis). Atherosclerosis can cause high blood pressure, which if not treated, can lead to blood vessel damage, stroke, heart failure, heart attack, or kidney failure.

It is well recognized that DM is characterized by enhanced up-regulation of the local and systemic Renin-Angiotensin-Aldosterone System (RAAS). Although the basis for dysfunction of the RAAS system in the setting of DM remains incompletely understood, its activation during DM has been demonstrated to be associated with increased oxidative damage which in turn activates the death pathwaysimplicated in myocardial cell apoptosis and necrosis. These myocyte and non-myocyte alterations in diabetic hearts resulting from increased activation of RAAS induce impairment of ventricular function. The benefits of RAAS blockade in preventing and reversing diabetic cardiomyopathy in DM patientsunderscore the importance of dysregulated RAAS in the pathogenesis of diabetic cardiomyopathy.

Renin-angiotensin system (RAS) inhibitors can reduce tissue Ang II levels, with beneficial effects on cardiovascular function. Therefore, blockade of the RAS may have the function of protecting against diabetic cardiomyopathy through inhibiting excessive activity of RAS. However, this has not been confirmed.

Alpha-lipoic acid (ALA) is a naturally occurring compound and is a cofactor of key metabolic enzyme complexes catalyzing the decarboxylation of alpha-keto acids. It has long been known as an essential cofactor for mitochondrial bioenergetic enzymes. Moreover, it has been identified as a powerful antioxidant found naturally in our foods such as spinach, broccoli, peas and meats. It appears to have increased functional capacity when given as a supplement in the form of a natural or synthetic isolate. Alpha Lipoic acid has been shown to combat oxidative stress by quenching a variety of Reactive Oxygen Species (ROS). Because this molecule is soluble in both aqueous and lipid portions of the cell, its biological functions are not limited solely to one environment.

Alpha-lipoic acid (ALA) exerts powerful protective effects in various cardiovascular disease models. However, its role in protecting against diabetic cardiomyopathy has not been elucidated.

So the present study was designed to detect the effect of AT II receptor antagonist (lozartan) and antioxidant (alpha lipoic acid) on blood glucose level, apoptosis, oxidative stress, serum lipid profile level, heart rate and MSBP in diabetic rats.

Materials and Methods

Animals:
This study was conducted on 50 adult Wistar albino male rats 6-8 weeks old, weighing between 170 and 200 g. Animals were housed in the animal laboratory at the medical research center at Benha faculty of medicine. They were housed at room temperature (25°C). All rats were fed a standard diet and water.

Groups of the experiment:
The animals were randomly divided into 5 groups each consisted of 10 rats as follow:
Group (1): Control group injected with a single dose of 1 ml citrate buffer, intraperitoneal (IP) and injected with 1 ml saline daily for 8 weeks.
Group (II): Diabetic group that received 40 mg/kg streptozotocin (IP) single dose.

Group (III): Diabetic group received losartan potassium at dose 1mg/kg/day. Losartan potassium was dissolved in distilled water and was given orally. It was administrated once the rats became diabetic for 8 weeks.

Group (IV): Diabetic group received alpha lipoic acid at a dose of 100 mg /kg (IP) 5 times a week. Alphalipoic acid dissolved in saline. It was administrated once the rats became diabetic for 8 weeks.

Group (V): Diabetic group received both losartan potassium at dose 1mg/kg/day orally and alpha lipoic acid at a dose of 100 mg /kg (IP) 5 times a week. Both were administrated once the rats became diabetic for 8 weeks.

Induction and diagnosis of diabetes mellitus:
Diabetes was induced by intraperitoneal (IP) injection of a single dose of STZ (40 mg/kg in freshly prepared citrate buffer pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. Control rats were injected by the buffer alone. Diabetes was verified 72 hours later by measuring blood glucose levels (after an overnight fasting) by tail blood glucose measurement with the use of glucose oxidase reagent strips. Rats having blood glucose level of ≥ 250 mg/dl were considered to be diabetic.

Chemicals used:
*Streptozocin drug:* It was purchased from Sigma- Aldrich Company (USA). It is presented in powder form, purity more than 99% to be dissolved in freshly prepared sodium citrate buffer pH 4.5.

*Sodium citrate buffer pH 4.5:*
Preparation of 0.1ml Citrate Buffer:
Weigh accurately citric acid 10.5 gm and sodium citrate 14.7 gm. Mix it with 500 ml water. Make up volume to 1000 ml with distilled water. Adjust pH 4.5 by sodium hydroxide.

*alpha lipoicacid:* It was purchased from Sigma Aldrich. In the form of Yellow to Yellow-Brown Powder.
*losartan potassium:* It was available in the form of losartan potassium film coated tablets (50 mg) from Unipharma.

Procedure of the experiments:
Measurement of MSBP (Cuff tail blood pressure method)
The rats were placed in the holder at least 10 to 15 minutes prior to obtaining pressure measurements for acclimatization. Proper animal handling is critical to consistent and accurate blood pressure measurements. A nervous, stressed animal may have diminished circulation in the tail. The animal was allowed to enter the holder freely. After the animal is in the holder, the nose cone was adjusted so the animal is comfortable but not able to move excessively. The cuff was applied to the tail and connected to the transducer and switched on to record the systolic and diastolic waves. From the charts the heart rate was calculated, the systolic and diastolic blood pressure values were measured then MSBP was calculated. Blood pressure was measured at the beginning and at the end of the experiment.

Blood sample collection
At the end of the treatment period, all animals were anesthetized with diethyl ether. The animals were fixed on operating table and the blood samples were taken as follow:
A craniocaudal incision of about 2 cm is made, parallel and with slightly to the left of the sternum through the skin and pectoral muscles to expose the ribs. A blunt curved forceps is then binged between the 5th and 6th ribs, through the intercostals muscles. The gap is widened so that the rapidly beating heart becomes visible, then the blood sample were taken from the right ventricle. Blood sample was collected in two tubes:

- One containing EDTA then immediately centrifuged for 15 min for separation of plasma and stored at – 20°C for estimation of plasma glucose.
- The other tube in which blood was left until clotting then centrifuged for 15 min for separation of serum and stored at – 20°C for estimation of total cholesterol and triglycerides.

Plasma concentration of glucose; serum triglyceride and total cholesterol were determined by a standard automated technique using Hitachi Analyzer Model 911 and adequate kits from Roche Company (Switzerland).
**Tissue preparation**
The heart was dissected and divided into two parts, one part for homogenization and measurement of MDA, GSH and caspase-3 activity. The rest of the heart fixed in 10% formalin solution at room temperature. Slices of cardiac tissue were processed for histopathological & immunohistochemical studies.

**Assessment of apoptosis**
Paraffin embedded tissue sections of 5 Mm were prepared on positively charged slides to be stained with anti BCL-X antibody using Biotin streptavidin immune-peroxidase technique\(^{28}\).

**Interpretation of immunostaining**
BCL-X was detected as cytoplasmic brown staining in examined tissue. Stained sections were classified as: mild intensity of apoptosis for weak brown cytoplasmic stain. Moderate intensity for moderate brown cytoplasmic stain. Severe intensity for strong brown cytoplasmic stain\(^{28}\).

**Measurement of antioxidant activity and apoptosis**
The tissues were separately weighed and homogenized in 10 volumes of cold 0.01 M Tris-HCL buffer (pH 7.4), using an automatic homogenizer. The homogenates were then centrifuged at 15,000 rpm for 15 min at 4 °C. Clear supernatants were used for malondialdehyde (MDA) assay as an indicator for lipid peroxidation and glutathione peroxidase (GSH-PX) as an antioxidant enzyme. In addition used for Caspase-3 enzymatic assay as an indicator for apoptosis. Tissue MDA level were determined by the thiobarbituric acid method and expressed as nmol/g tissue\(^{21}\). Glutathione peroxidase (GSH-PX) activity was measured by NADPH oxidation and expressed as ug/gm tissue\(^{21}\). Caspase-3 activity was measured by Colorimetric Assay Kit from (Bioscience, USA) and expressed as units/ mg\(^{3}\).

The measurements were done in the biochemistry analyzing unit at Benha faculty of medicine.

**Statistical analysis:**
All data were expressed as mean ± S.D; data were evaluated by the one way analysis of variance using computer with SPSS version 19 program. Difference between groups were compared by Student's t-test with \(P < 0.05\) selected as the level of statistical significance.

**Results**
**Mortality rate:**
No rats died in the control group and in the group that received losartan and alpha lipoic acid. Whereas three rats died in the non-treated STZ group, with a mortality rate of 30%. Among the drug-treated STZ groups, one rat died in the group receiving losartan and one died in the group receiving alpha lipoic acid with a mortality rate of 10% in either group.

**Biochemical parameters:**
Table 1 showed that there was significant increase (\(P< 0.001\)) in plasma glucose level in group II (diabetic group) compared to the control. The serum triglycerides and total cholesterol were significantly increased (\(P<0.001\)) in group II compared with the control. Losartan administration in diabetic rats (group III) caused significant decrease (\(P< 0.001\)) in blood glucose triglycerides and total cholesterol levels compared to the diabetic rat. Alpha lipoic acid administration in diabetic rats (group IV) caused significant decrease (\(P< 0.001\)) in blood glucose, triglycerides and total cholesterol levels compared to the diabetic rat.

**Table 1:** Comparison of blood glucose level (mg/dl), triglyceride (mg/dl) and cholesterol level (mg/dl) among the experimental groups (mean ± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose level (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Control group n=10</td>
<td>167.9±2.13</td>
<td>73.5±3.2</td>
<td>89.4±1.96</td>
</tr>
<tr>
<td>II: STZ-Diabetic rats n=7</td>
<td>523.57±9.6*</td>
<td>236.6±5.98*</td>
<td>248.4±3.86*</td>
</tr>
<tr>
<td>III: Diabetic rats &amp; Losartan n=9</td>
<td>317.11±3.5*</td>
<td>107.46±4.5*</td>
<td>125.7±4.1*</td>
</tr>
<tr>
<td>IV: Diabetic rats &amp; alpha lipoic acid n=9</td>
<td>229.6±3.8*</td>
<td>96.3±2.6*</td>
<td>107.6±4.4*</td>
</tr>
</tbody>
</table>
Heart rate and blood pressure changes:
Table 2: showed that there was significant decrease of the heart rate and increase in MSBP in STZ induced diabetic rats compared to the control group. Administration of losartan and alpha lipoic acid leads to significant increase of heart rate and decrease of MSBP compared to the diabetic group (group II).

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart rate beat /min</th>
<th>Mean systemic blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>253±2.8</td>
<td>75.95±1.27</td>
</tr>
<tr>
<td>II</td>
<td>117.4±1.7*</td>
<td>158.4±2.56*</td>
</tr>
<tr>
<td>III</td>
<td>239.1±3.8@</td>
<td>98.66±1.6@</td>
</tr>
<tr>
<td>IV</td>
<td>237±3.7*</td>
<td>117.67±3.04*</td>
</tr>
<tr>
<td>V</td>
<td>253.6±1.59**</td>
<td>95±1.6**</td>
</tr>
</tbody>
</table>

*Significant difference (P<0.001) compared with the control group (group I).
@ Significant difference (P<0.001) compared with the diabetic group (group II).
& Significant difference (P<0.001) compared with (group III).
$ Significant difference (P<0.001) compared with (group IV).

Oxidative, antioxidative and apoptosis changes in cardiac tissue:
Table 3: showed the effect of Losartan and alpha lipoic acid on antioxidative activity and apoptosis in the cardiac tissue homogenate. There was significant increase of MDA and caspase -3 and decrease of GPX level in STZ induced diabetic rats (group II) compared to the control group (group I). Both losartan and alpha lipoic acid treatment caused significant decline (P<0.001) in MDA and caspase -3 with significant increase in GSH-PX level in cardiac tissue homogenate.

Table 3: Comparison of the heart rate beat /min and mean systemic blood pressure (MSBP) mmHg among the experimental groups (mean ± SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Apoptosis index</th>
<th>Antioxidant parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cardiac caspase -3 activity (U/mg tissue)</td>
<td>MDA (nmol/gm tissue)</td>
</tr>
<tr>
<td>Group I: Control group n=10</td>
<td>4.65±0.07</td>
<td>2.15±0.06</td>
</tr>
<tr>
<td>Group II: STZ-Diabetic rats n=7</td>
<td>195.1±4.3*</td>
<td>80.5±2.3**</td>
</tr>
<tr>
<td>Group III Diabetic rats&amp; Losartan n=9</td>
<td>239.1±3.8@</td>
<td>98.66±1.6@</td>
</tr>
<tr>
<td>Group IV: Diabetic rats&amp; alpha lipoic acid n=9</td>
<td>237±3.7*</td>
<td>117.67±3.04*</td>
</tr>
<tr>
<td>Group V: Diabetic rats&amp; Losartan&amp;alpha lipoic acid n=10</td>
<td>253.6±1.59**</td>
<td>95±1.6**</td>
</tr>
</tbody>
</table>

*Significant difference (P<0.001) compared with the control group (group I).
@ Significant difference (P<0.001) compared with the diabetic group (group II).
& Significant difference (P<0.001) compared with (group III).
$ Significant difference (P<0.001) compared with (group IV).
**Table 1:**

<table>
<thead>
<tr>
<th>Group II: STZ-Diabetic rats n=7</th>
<th>11.79±0.24*</th>
<th>5.49±0.14*</th>
<th>176.2±0.94*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III: Diabetic rats &amp; Losartan n=9</td>
<td>9±0.2*</td>
<td>4.13±0.09*</td>
<td>209.3±1.3*</td>
</tr>
<tr>
<td>Group IV: Diabetic rats &amp; alpha lipoic acid n=9</td>
<td>6.3±0.2*</td>
<td>3.4±0.13*</td>
<td>237.3±2.6*</td>
</tr>
<tr>
<td>Group V: Diabetic rats &amp; Losartan &amp; alpha lipoic acid n=10</td>
<td>5.59±0.12*#&amp;§</td>
<td>2.4±0.07#&amp;§</td>
<td>256.9±2.1#&amp;§</td>
</tr>
</tbody>
</table>

*Significant difference (P<0.001) compared with the diabetic group (group II).

@ Significant difference (P<0.001) compared with the diabetic group (group III).

$ Significant difference (P<0.001) compared with the diabetic group (group IV).

**Apoptosis in cardiac tissue:**

Fig. 1 showed normal cardiomyocytes with mild brown staining of BCL-X immuno-histochemistry indicating mild degree of apoptosis (control group). Fig. 2 showed strong brown staining of cardiomyocytes; so that there was an increase in apoptotic cell death in myocardium in diabetic rats (group II). Fig. 3 showed moderate brown staining of cardiac muscle indicating moderate improvement of apoptotic changes in diabetic rats receiving losartan treatment (group III). Fig. 4 showed weak brown staining of cardiac muscle indicating strong improvement of apoptotic changes in diabetic rats after alpha lipoic acid administration (group IV). Fig. 5 also showed weak brown staining of cardiac muscle indicating marked improvement of apoptotic changes in diabetic rats after combined administration of alpha lipoic acid and losartan (group IV).

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![Section in myocardial muscles of normal (group I) rats showing weak BCL-X expression in cytoplasm of myocardial muscles (strept – avidin – biotin) ×200.](image-url)
Fig. 2: Section in myocardial muscles of diabetic rat (group II) showing STRONG BROWN BCL-X STAINING in cytoplasm of myocardial muscles (strept – avidin – biotin) x200.

Fig. 3: Section in myocardial muscles of diabetic rat received lozartan (group III) showing moderate BROWN BCL-X STAINING in cytoplasm of myocardial muscles (strept – avidin – biotin) x200.
DISCUSSION

The results obtained from this study demonstrate that apoptosis significantly increased in diabetic myocardium and provide evidence that high levels of glucose directly cause apoptosis. Our study has identified that diabetic myocardial apoptosis is associated with oxidative overactivity as evident by significant increased MDA level.
indicating increased lipid peroxidation in the cardiac tissue and significant decreased in GSH-PX level in cardiac tissue indicating decreased antioxidative activity. In agreement with Roy S who suggested that hyperglycemia directly induces apoptotic cell death in the myocardium in vivo. Hyperglycemia-induced myocardial apoptosis is mediated, at least in part, by activation of the cytochrome c-activated caspase-3 pathway, which may be triggered by ROS derived from high levels of glucose. CaiL et al identified that mitochondrial cytochrome c release and caspase-3 activation is associated with hyperglycemia-induced myocardial apoptosis. Hyperglycemia was also able to induce apoptotic cell death in neuron cells in vivo and in vitro and endothelial cells in vitro by activating caspase-3, a downstream pivotal step to initiate apoptosis. The correlation between ROS production and mitochondrial cytochrome c release-mediated caspase-3 activation suggests that ROS derived from high levels of glucose may trigger the apoptotic process. Mitochondria are a major source of ROS production. Cellular sources of ROS generation within the heart include cardiac myocytes, endothelial cells and neutrophils. ROS leads to cellular damage through several mechanisms (oxidation, interference with nitric oxide [NO] and modulation of detrimental intracellular signaling pathways). Therefore, increased ROS causes cardiac dysfunction by direct damage to proteins and DNA, and by inducing apoptosis.

Diabetic cardiomyopathy and its mechanisms: Role of oxidative stress and damage

Quan Liu1, Shudong Wang1,2 and Lu Cai2,3,*
Journal of Diabetes Investigation J Diabetes Invest Vol. 5 No. 52014

Our finding showed that STZ induced diabetic rats have significant decreased in heart rate this in agreement with 20, who demonstrated that conscious chronic diabetic rats presented lower heart rate variability than control rats. In addition our study showed that diabetes induced by STZ injection leads to significant increase in mean systemic arterial blood pressure. This result was in agreement with Stephen O. et al 22; Stem N and Tuck M 23 who revealed that STZ injected rats tend to be hyperphagic, therefore consuming more sodium than their nondiabetic control group, and there is evidence that blood pressure in IDDM may be salt sensitive. Other possibility is that the blood pressure increase is due to changes in intravascular volume. Blood volume has been proposed to increase with diabetes, because of hyperglycemia-induced osmotic fluid shifts. In addition Olivier R et al 24 revealed that diabetes induced hypertension caused by oxidative stress, inflammation, increased sympathetic nervous activity and up-regulation of rennin angiotensin system as well as Metabolic abnormalities associated with DM impair the integrity of elastic fibres in the arterial wall which translates into increased stiffness of the wall material. The accumulation of advanced glycation end-products (AGEs) is a major pathogenic mechanism contributing to arterial stiffening in DM 10.

Our results showed that STZ injection leads to significant increased in blood glucose, triglyceride and total cholesterol level this along with Vergès B 25 who stated that untreated type 1 diabetes show hypertriglyceridaemia, this is mainly due to decreased lipoprotein lipase (LPL) activity as the insulin is a potent activator of the LPL, which promote the catabolism of triglyceride rich lipoproteins and reducing as a consequence the plasma triglyceride levels. Insulin not only enhances LPL activity, but also has a direct, positive effect on the LPL gene to promote LPL synthesis, while hypercholesterolemia in STZ-induced diabetic rats results from increased intestinal absorption and synthesis of cholesterol.

In our study administration of lozartan caused significant decreased in blood glucose level, this agreed with Yang and Peng XD 26 as they demonstrated that the clinical use of angiotensin converting enzyme (ACE) inhibitors has been associated with increased insulin sensitivity and ACE inhibitors modulate the early steps of insulin signaling. Other mechanism explain the hypoglycemic effect of lozartan has been reported by Chan P et al 27 who stated that the treatment with angiotensin receptor blocker (ARB) decreased the plasma glucose level in STZ diabetic rats due to partial inhibition of the sodium-glucose cotransporters (SGLTs) in the renal proximal tubular cells leading to decrease the glucose reabsorption in the renal tubules, in addition to enhancing the glucose utilization in peripheral tissues and reduction of hepatic gluconeogenesis via non-insulin mediated mechanisms. Tikellis C et al 28 also reported that there was an increase in β-cell mass of the pancreas after losartan treatment and this could be due to increased proliferation rates of the residual β-cells that escaped the STZ toxic effect, β-cell differentiation from exocrine progenitors (neogenesis), a reduction in β-cell apoptotic rates, or the combined action of the all these 3 mechanisms.

Our results showed decreased in myocardial apoptosis indexed by significant decrease in caspase-3 activity and decreased in myocardial lipid peroxidation indexed by significant decrease in MDA level and increased anti-oxidant enzymes indexed by significant increase in GSH-PX level in cardiac tissue after losartan administration. These results were in agreement with Vivek P. et al 29 who reported that ROS generation by exposure to high levels.
of glucose was found in the myoblast cells and indicated that suppression of angiotensin II can inhibit diabetic cardiomyopathy through inhibition of oxidative stress and myocardial cell death. Other study suggested that Myocyte apoptosis occurs by upregulation of any component of the RAS from angiotensinogen to angiotensin receptor level. AT2 linked death cascade requires tyrosine phosphatase that promotes ceramide synthesis. Elevation of intracellular ceramide proceeds caspase-3 activation. It is noted that apoptosis is mainly mediated by activation of caspase-3. Moreover, Ang II stimulates the release of aldosterone that also stimulates cardiomyocyte apoptosis by caspase 3 activation.

Furthermore; our results showed significant increased of heart rate to the normal level and significant decreased of MSBP, serum triglyceride and serum cholesterol level in lozartan received rats. This was in agreement with kyvelou g et al and Erik s et al who reported that administration of losartan potassium in diabetic rats resulted in decrease of triglycerides and total cholesterol, the lipid lowering property of ARB (angiotensin receptor blockers) as they suggested that some ARB activateperoxisome proliferator-activated receptor- gamma(PPAR-γ) which is involved in the regulation of carbohydrate and lipid metabolism. Furthermore, there is increasing evidence that PPAR-γ agonists exert anti-inflammatory, antioxidative, and antiproliferative effects on the vascular walland reduce triglycerides and LDL levels.

Our results revealed that; administration of ALA to diabetic rats caused significant decrease in plasma glucose level, triglyceride and total cholesterol level. These results coincide with results of Thirunavukkarasu V et al who revealed that ALA supplementation significantly reduced plasma total cholesterol, low density lipoprotein-cholesterol (LDL-C) and triglycerides and increased high density lipoprotein-cholesterol (HDL-C) in STZ-diabetic rats. The mechanism by which ALA improves the dyslipidemia is still unclear. One of the actions which might attribute to this finding is by decreasing the non-esterified fatty acid levels. In diabetes there is deficiency of lipoprotein lipase activity that contributes significantly to the elevation of triglycerides. Improving the blood glucose levels served to lower plasma triglycerides levels by returning lipoprotein lipase levels to normal. In addition, Nouf M. et al explained that in skeletal muscle, ALA is proposed to recruit the glucose transporter GLUT4 from its storage site in the Golgi to the sarcolemma, so that glucose uptake is stimulated by the local increase in transporter abundance. ALA enhances glucose uptake by rapid translocation of the glucose transporters GLUT1 and GLUT4 from an internal membrane fraction to the plasma membrane improving insulin action. In addition, ALA has been shown to enhance glucose oxidation in diabetic subjects.

Our results revealed that administration of ALA to diabetic rats caused significant decrease in MDA and caspase-3 with significant increase in GSH-PX in cardiac tissue. These results go along with the results of Siti Bet al who found that ALA prevents oxidation injury and cell death by several mechanisms, such as scavenging oxygen radicals, protecting against lipid peroxidation and chelating metal ions. Supplementation of ALA was shown to be capable of protecting the majority of pancreatic islet cells from damage due to STZ cytotoxicity. Zhang Y et al found that in STZ-diabetic rats ALA supplementation significantly restored MDA and GSH levels in cardiac tissues and explained the decrease in MDA by the decrease in the oxidation of lipid. Concerning the increase in GSH-PX Petersen S et al revealed that ALA enters the cell and is reduced to dihydrolipoic acid (DHLA) by GSH reductase and thioredoxin reductase. Higher levels of GSH were expected in ALA rats because ALA and DHLA act as a redox couple that can regenerate other natural antioxidants such as GSH-PX, vitamin C, coenzyme Q10, and vitamin E thereby protecting the integrity of cell membrane. Also they predicted that ALA is able to elevate GSH levels through its ability to increase cysteine uptake which is the precursor of GSH synthesis and subsequently increase the de novo synthesis of GSH. Our results revealed that administration of ALA to diabetic rats caused significant decrease in caspase-3 activity in cardiac tissue. This result go along with Chun Li et al as they found that mitochondrial cytochrome c release was significantly suppressed by antioxidant treatment, and down-regulation of caspase-9, -3. These changes were accompanied by decreasing cardiac cell apoptosis. As well as Woo J et al reported that the increase in apoptosis and caspase activities were all inhibited by 0.5 mmol/L ALA in obese rats.

Our results revealed that alpha lipoic acid decreased mean systemic arterial blood pressure and increased heart rate in diabetic rats. These results go along with Thyago M et al who revealed that, the results from the present study and those from previous findings support the insight that oxidative stress reduces baroreflex sensitivity and that anti-oxidant therapy, such as α-lipoic acid, could restore it. As well as Galan C. et al found that ALA improves endothelial NO synthesis and thus improving endothelial function and improve renovascular hypertension.

In our study combination of alpha lipoic acid and losartan leads to significant protection than each one alone. Thus, cardiac muscle apoptosis represents a novel therapeutic target for the control of diabetic cardiomyopathy in response to therapeutic agents as losartan which blocked angiotensin II receptors and alpha lipoic acid through its antioxidant mechanism.
ACKNOWLEDGMENT
Our deep gratitude and appreciation to Dr. Ayman M. Mousa lecturer of histology, Faculty of Medicine, Benha University for his great help and valuable constructive cooperation in the histopathological part.

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