

The Effect of Alpha Lipoic Acid and Melatonin on the progression of Streptozotocin-induced diabetic cardiomyopathy in rats

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Abstract

Background: Diabetic cardiomyopathy (DCM) is a major cause of diabetes-related morbidity and mortality. Alpha lipoic acid (ALA) and Melatonin (Mel) have gained a considerable amount of attention as antioxidants, their effect on the progression of DCM has not yet determined. **Aim:** To evaluate effects of ALA and Mel on AMP-activated protein kinase (AMPK) activity and the state of oxidative stress (OS) in DCM in rats. It also aims at correlating the pathogenesis of DCM to AMPK activity. **Methods:** 60 rats were divided into 6 groups (n=10); control (CG), C + ALAG, C + MelG, Diabetic (DG), D + ALAG and D + MelG. Diabetes was induced by 60mg/Kg streptozotocin. ALA and Mel were given in a dose of 100 mg/kg/day and 10 mg/kg/day respectively for 12 weeks. The heart/body weight (Ht/BW) ratio, AMPK activity and oxidative state in cardiac tissue, serum cardiac enzymes (serum lactate dehydrogenase "LDH" and creatine kinase "CK"), lipid profile, fasting glucose level and histologic examination were assessed at the end of the study. **Results:** Ht/BW and OS increased in DG compared to CG, decreased subsequently by ALA and Mel treatment with no significant difference between both groups. LDH and CK were higher in DG as compared to CG. Cardiac AMPK activity was decreased in DCM and increased subsequently by ALA and Mel treatment. Normal cardiac architecture was restored by both of them. ALA and Mel showed a hypolipidemic effect while ALA had a hypoglycemic effect. **Conclusions:** ALA and Mel enhanced AMPK activity, and antioxidant activity in the heart thus decreased the progression of cardiac dysfunction.

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Keywords

- Diabetic cardiomyopathy
- Alpha lipoic acid
- Melatonin
- AMPK
- oxidative stress.

Introduction

Cardiomyopathies are diseases of the heart muscle that are associated with cardiac dysfunction and heart failure and thus they represent a major health problem. The phenotype-based classification describes the major forms of cardiomyopathy, but not their causes, where cardiomyopathies, after the statement of ESC Working Group on Myocardial and Pericardial Diseases, are classified into; hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC)/arrhythmogenic ventricular cardiomyopathy, and left ventricular non compaction (LVNC)(1). Defined causes of different types of cardiomyopathy are still unknown but cardiomyopathies can be either primary myocardial disorders or develop as a secondary consequence of a variety of conditions, including myocardial ischemia, inflammation, increased myocardial pressure or volume load, and diabetes. Diabetic cardiomyopathy describes diabetes-associated changes in the structure and function of the myocardium that are not directly attributable to other confounding factors such as coronary artery disease (CAD) or hypertension. The concept of diabetic cardiomyopathy was first introduced by Rubler *et al*(2), and has subsequently been widely used by epidemiologists and clinicians. Several mechanisms have been implicated in the pathogenesis of diabetic cardiomyopathy. Changes in myocardial structure, calcium signaling and metabolism are early defects that have been described mainly in animal models and may precede clinically manifest cardiac dysfunction(3).

However a better understanding of its pathophysiology is necessary for early diagnosis and the development of treatment strategies for diabetes-associated cardiovascular dysfunction. AMP-activated protein kinase (AMPK), a heterotrimeric enzyme, is a key regulator of cellular energy metabolism consisting of the catalytic α -subunits ($\alpha 1$ or $\alpha 2$), β -regulatory subunits ($\beta 1$ or $\beta 2$) and AMP binding subunits ($\gamma 1$, $\gamma 2$ or $\gamma 3$)(4). It plays a key role as a master regulator of cellular energy homeostasis. The kinase is activated in response to stresses that deplete cellular ATP supplies such as low glucose, hypoxia, ischemia, and heat shock(5). When cells are exposed, for example, to low oxygen concentration, they readily “respond” by inducing adaptive reactions for their survival through the AMPK pathway(6). AMPK can switch on other ATP-generating pathways such as glycolysis and amino acid oxidation, while simultaneously switching off ATP-utilizing pathways such as fatty acid synthesis and gluconeogenesis(7). Recently AMPK was proved to be also activated by oxidative stress(8). On the other hand, dysfunctional AMPK activity is related to the decrease of antioxidant gene expression and the increase of oxidants' production resulting in oxidative stress development(9). Oxidative stress has been linked to the onset of diabetes (10). It plays a pivotal role in the development of its complications, both microvascular and cardiovascular. Several studies have shown that oxidative damage induced by reactive oxygen or nitrogen species (ROS and/or RNS) derived from hyperglycemia plays a critical role in diabetic injury in multiple organs(11). Alpha lipoic acid (ALA) and melatonin are naturally occurring

compound; ALA is involved in mitochondrial dehydrogenase reactions and has recently gained a considerable amount of attention as a novel antioxidant. The beneficial role of ALA in improving diabetic neuropathy has been extensively studied and clinically approved(12). Melatonin is a pleiotropic molecule(13) with antioxidative, antifibrotic properties and cell regenerative capacity in liver(14), heart(15), skin flaps(16),and brain(17). ALA and melatonin have been successfully employed in a variety of in vivo models: ischemia–reperfusion, heart failure, and hypertension(18,19). But their effect on the development and progression of DCM has not yet determined. This study aimed to evaluate the potential effects of ALA and melatonin on AMPK activity and the state of oxidative stress in streptozotocin-induced DCM in rats.

Subjects and methods:

This study was carried out on 60 male albino rats (200 to 250 g), which were housed in an animal facility at Faculty of Medicine, Alexandria University. They were maintained at room temperature ($25\pm 2^{\circ}\text{C}$) under standard conditions of light–dark cycle with free access to rat chow and water. All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (1985), NIH, Bethesda, and approved by the Ethics Committee of Faculty of Medicine, Alexandria University.

Study design: Rats were divided into 6 groups (10 rats/group); **CG**, normal control non diabetic group. **C+ALAG**, non diabetic control rats that were given ALA in a dose of 100 mg/kg/day I.P injection for 12 weeks. **C+MelG**, non diabetic control rats that were given melatonin in a dose of

10 mg/kg/day intraperitoneal (I.P) injection for 12 weeks. **DG**, streptozotocin-induced diabetic group and did not receive any treatment during the study period. **D+ALAG**, streptozotocin-induced diabetic group and were given ALA in a dose of 100 mg/kg/day I.P injection for 12 weeks. **D+MelG**, streptozotocin-induced diabetic group and were given Mel in a dose of 10 mg/kg/day I.P injection for 12 weeks.

Induction of diabetes: Diabetes was induced by single I.P injection of 60mg/Kg streptozotocin. Rats with fasting blood glucose level above 300mg/dL were diabetic and included in the study.

Blood and heart Collection: The day of sacrifice, rats were weighed then euthanized by deep anesthesia with 100 mg/kg pentobarbital. The chest wall was opened using scissors to expose the heart, which was then removed and immediately weighed for calculating the heart/body weight ratio. The heart tissues were divided into two halves. One half was then stored at -80°C until assay and the other one was fixed in 10% formalin for histological examination. The blood was collected from the aorta with EDTA-treated syringe needles, and immediately mixed with 3.2% sodium citrate at a ratio of 9:1 (blood volume/citrate volume). It was then centrifuged at $1,500 \times g$ for 15 minutes to separate the plasma that was frozen in aliquots at -80°C until assay.

AMPK activity: The heart tissue samples were homogenized, one half in a buffer containing 30mM tris-Hcl, 150Mm Nacl, 1mM EDTA, 1mM EGTA, 1% Triton X-100and 0.5ML/ml of protease inhibitor cocktail F (Bio Basic Inc, Canada). The homogenate was then centrifuged at $100,000 \times g$

(Beckman ultracentrifugation) for 45 minutes at 4°C and supernatants were collected and stored at -20°C for determination of AMPK concentration by dot immunoblot assay. The other half of heart tissue was homogenized in cold 1 x PBS and centrifuged for 5 minutes at 5,000rpm and the clear supernatants were stored at -20°C for assay of total antioxidant capacity and hydrogen peroxide concentration(20). Protein content was assayed using Lowry method(21).

Dot immunoblot assay: The AMPK concentration was determined by spotting of equivalent concentrations of protein sample into nitrocellulose filter, and then incubated in blocking solution for 1 hour at room temperature. The filters were incubated with the AMPK antibody (R&D system, UK) in concentration of 1µg/ml for 1 hour. Then the blot was washed three times (5minutes each) with 1x PBST (0.05% Tween in phosphate buffered saline PBS) and then washed for 10 minutes with distilled water with shaking. Filters were then incubated with secondary antibodies (Koma Biotech Inc, Korea) at a dilution of 1:2000 for 1hour. After the secondary incubation, the membrane was washed 3 times (5 minutes each) with PBST (0.05% Tween) and then washed again in distilled water with shaking. 3,3'-Diaminobenzidine (DAB) substrate solution was prepared, and then 30 µl hydrogen peroxide were added. After developing the color of the blot, the reaction was stopped after appearance of the expected bands by pouring out the substrate and rinsing with distilled water repeatedly. Finally the membrane was dried and placed in the dark and pictures were taken. The pictures were fed to the computer using the Corel paint shop pro X2

software, the color intensity of each band was converted to a number with red green blue (RGB) unit and divided by the protein concentration in each sample to be represented finally with RGB/mg protein(20).

Markers of oxidative stress: Parameters of oxidative stress were assayed by colorimetric technique using commercial kits (Biodiagnostic, Egypt) according to the manufacturer instructions. The protein content of the supernatants was determined using Lowry's method(21). Oxidative damage parameter, hydrogen peroxide (H₂O₂) (nmol/gm tissue) and total antioxidant capacity (TAC) (mmol/L)(22) were assayed in tissue lysate. **Cardiac enzymes:** Serum Creatine Kinase (CK) activity was assessed using the Creatine Kinase Kit from BioSystems S.A. and kinetic UV method according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) specifications (COD 11790). Serum lactate dehydrogenase (LDH) activity was assayed using the colorimetric kinetic method and the QuantichromTM Lactate Dehydrogenase Kit (DLDH-100) from BioAssay Systems USA.

Lipid Profile and Fasting Blood Glucose: Lipid profile including total-cholesterol, Triglycerides, LDL-cholesterol and HDL-cholesterol were measured using colorimetric technique according to manufacture instructions (Biodiagnostic, Egypt). Fasting blood glucose (FBG) level was tested via tail vein prick using glucose-oxidase reagent strips (One Touch Ultra, Johnson & Johnson®, New Brunswick, NJ, USA). **Histologic Analysis:** Heart specimens were fixed with 10% buffered formalin overnight, embedded in paraffin,

sectioned for hemotoxylin and eosin (H&E) staining, and examined under light microscopy.

Results:

1. Effects of melatonin and ALA on heart/body weight ratio

The Ht/BW ratio increased significantly in diabetic group (DG) compared to the control group (CG) (0.40 ± 0.02 vs 0.26 ± 0.01 , $P<0.05$), it decreased subsequently by melatonin and ALA treatment, where the effect of melatonin was more announced than that of ALA (0.30 ± 0.02 vs 0.32 ± 0.01 , $p<0.05$). Noticing that the administration of ALA or melatonin in normal non diabetic rats (C+ALAG, C+MelG) did not affect the heart size when compared to NG (Table 1).

2. Effects of melatonin and ALA on CK and LDH

The levels of LDH, and CK were significantly increased in diabetic untreated rats compared with the normal control rats ($p\leq 0.05$). However, melatonin and ALA treatment markedly reduced the levels of CK and LDH. There was no difference in the effect of melatonin and ALA on cardiac enzymes in diabetic rats (Table 1).

3. Effects of Melatonin and ALA on AMPK level

Western blot analysis showed that cardiac AMPK

level was decreased in DG hearts (146.5 ± 4.5 RGB/mg protein) when compared to its level in normal non-diabetic rats (155.4 ± 1.7 RGB/mg protein). Melatonin and ALA treatment caused significant increase in its level (153.8 ± 13.7 and 155.0 ± 2.6 RGB/mg protein respectively) with no significant difference between the two latter groups (Table 1).

A significant negative correlation was found between AMPK and cardiac enzymes in all experimental groups. (Figure 1)

4. Antioxidant effect of Melatonin and ALA

Hydrogen peroxide, which reflects oxidant-induced lipid peroxidation reactions, was significantly increased in diabetic rats (DG) compared to normal control ones (CG) (111.3 ± 5.2 vs 103.5 ± 3.1 nmol/gm tissue). The antioxidant effect of melatonin and ALA was shown through the reduction of its level significantly in D+MelG and D+ALAG ($p\leq 0.05$). This was further confirmed by the results of the total antioxidant capacity in the studied groups, where TAC was decreased in DG compared to CG and significantly increased by melatonin and ALA treatment (Table 1).

A significant positive correlation between AMPK and TAC in addition to another negative correlation between AMPK and H_2O_2 were present in all studied groups. (Figure 2)

Table (1): Comparison between the different studied groups according to Ht/BW ratio, CK, LDH, AMPK activity, H₂O₂ and TAC.

	Control			Diabetic			p
	CG	C+ALAG	C+MelG	DG	D+ALAG	D+MelG	
Ht/BW	0.26±0.01	0.26±0.02	0.25±0.01	0.40 ^a ±0.02	0.30 ^{ab} ±0.02	0.32 ^{abc} ±0.01	<0.001*
CK (U/L)	214.4±64.3	224.9±47.6	212.7±46.5	383.6 ^a ±20.0	257.0 ^b ±62.2	223.9 ^b ±28.0	<0.001*
LDH (U/L)	320.3±61.0	338.1±54.3	338.6±59.1	525.3 ^a ±55.5	102.1 ^{ab} ±14.1	83.1 ^{ab} ±37.6	<0.001*
AMPK (RGB/mg protein)	155.4± 1.7	156.1± 1.6	156.2± 4.3	146.5 ^a ± 4.5	155.0 ^b ± 2.6	153.8 ^b ± 13.7	0.009*
H ₂ O ₂ (nmol/gm)	103.5±3.1	102.9±3.8	104.2±2.6	111.3 ^a ±5.2	98.2 ^{ab} ±6.3	99.7 ^b ±4.9	<0.001*
TAC (mmol/L)	2.4±0.1	2.2±0.2	2.4±0.1	1.7 ^a ±0.4	2.2 ^b ±0.2	2.3 ^b ±0.3	<0.001*

p: p value for F test (ANOVA), Significant between groups was done using Post Hoc Test (LSD). a: significant with CG, b: significant with DG, c: significant with D+ALAG, *: Statistically significant at p≤0.05.

5. Effects of Melatonin and ALA on Lipid profile

Lipid profile was impaired in streptozotocin-diabetic rats. The treatment with melatonin and ALA caused a significant reduction of the cholesterol, TG and LDL levels in diabetic rats. On the other hand, HDL was significantly increased compared to diabetic untreated group (DG) (Table 2).

6. Effects of Melatonin and ALA on FBG level

FBG level was significantly increased after the induction of diabetes. Treatment with melatonin

and ALA significantly decreased the FBG level when compared to the diabetic untreated group (DG). Yet FBG level in ALA-treated group was significantly lower than that treated with melatonin (Table 2).

7. Effect of melatonin and ALA in non-diabetic rats

Results showed that the melatonin and ALA have no influence on AMPK activity, oxidative status, lipid profile and FBG levels in the normal non-diabetic rats.

Table (2): Comparison between the different studied groups according to lipid profile (Cholesterol, TG, HDL, LDL) and Fasting blood Glucose (FBG)

	Control			Diabetic			P
	CG	C+ALAG	C+MelG	DG	D+ALAG	D+MelG	
Cholesterol (mg/dl)	89.6±1.7	89.8±1.8	90.1±2.0	189.8 ^a ±2.1	116.3 ^{ab} ±4.4	120.0 ^{abc} ±2.2	<0.001*
TG (mg/dl)	90.2±2.0	90.8±2.1	91.7±1.6	216.4 ^a ±5.4	108.4 ^{ab} ±2.2	110.4 ^{ab} ±1.1	<0.001*
HDL(mg/dl)	46.4±1.8	45.8±2.1	45.0±2.0	34.6 ^a ±2.0	42.4 ^{ab} ±1.6	43.3 ^{ab} ±2.9	<0.001*
LDL(mg/dl)	64.3±1.7	65.4±1.7	64.1±1.4	87.5 ^a ±1.3	69.9 ^{ab} ±1.7	69.9 ^{ab} ±1.3	<0.001*
FBG(mg/dl)	83.5± 6.5	83.5± 7.0	83.2± 7.0	423.5 ^a ± 43.2	319.1 ^{ab} ± 35.1	417.7 ^{ac} ± 49.6	<0.001*

p: p value for F test (ANOVA), Significant between groups was done using Post Hoc Test (LSD). a: significant with CG, b: significant with DG, c: significant with D+ALAG, *: Statistically significant at p≤0.05.

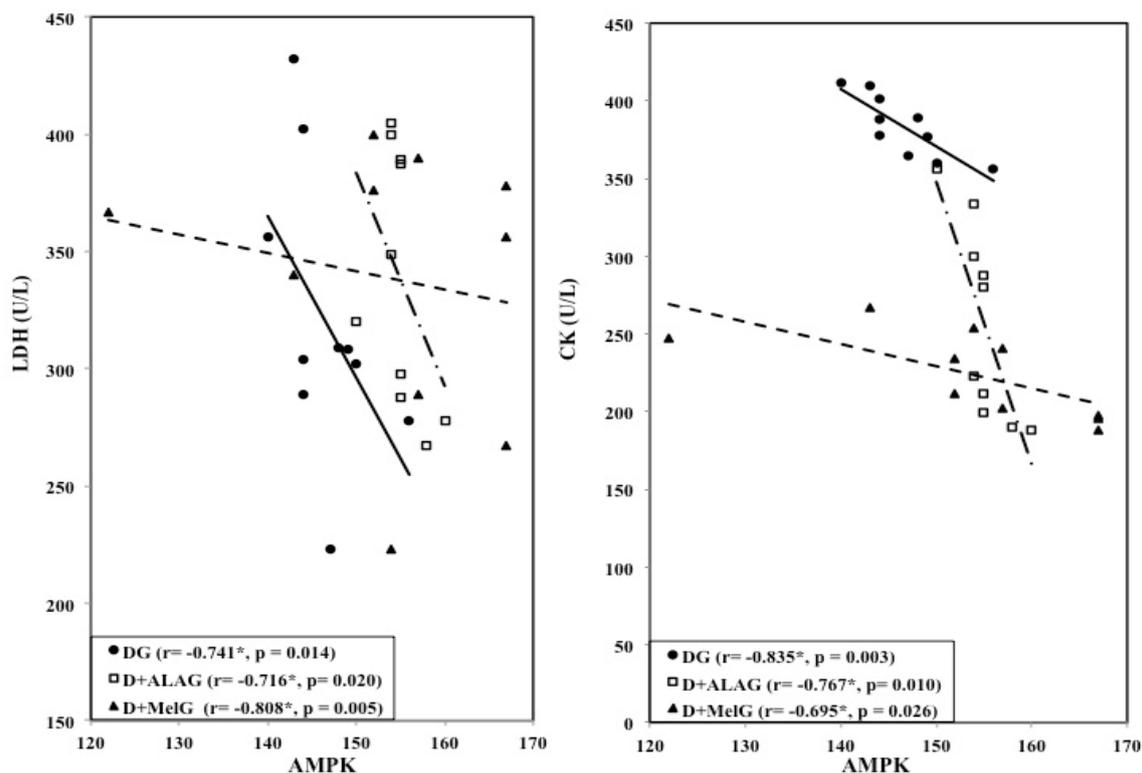


Figure 1; Correlation between AMPK activity and cardiac enzymes in the serum. **a;** AMPK is negatively correlated to the LDH in DG ($r = -0.741^*$, $p = 0.014$), D+ALAG ($r = -0.716^*$, $p = 0.020$) and D+MelG ($r = -0.808^*$, $p = 0.005$). **b;** AMPK is negatively correlated to the CK in DG ($r = -0.835^*$, $p = 0.003$), D+ALAG ($r = -0.767^*$, $p = 0.010$) and D+MelG ($r = -0.695^*$, $p = 0.026$). AMPK; AMP-activated protein kinase, CK; Creatine Kinase LDH; Lactate Dehydrogenase.

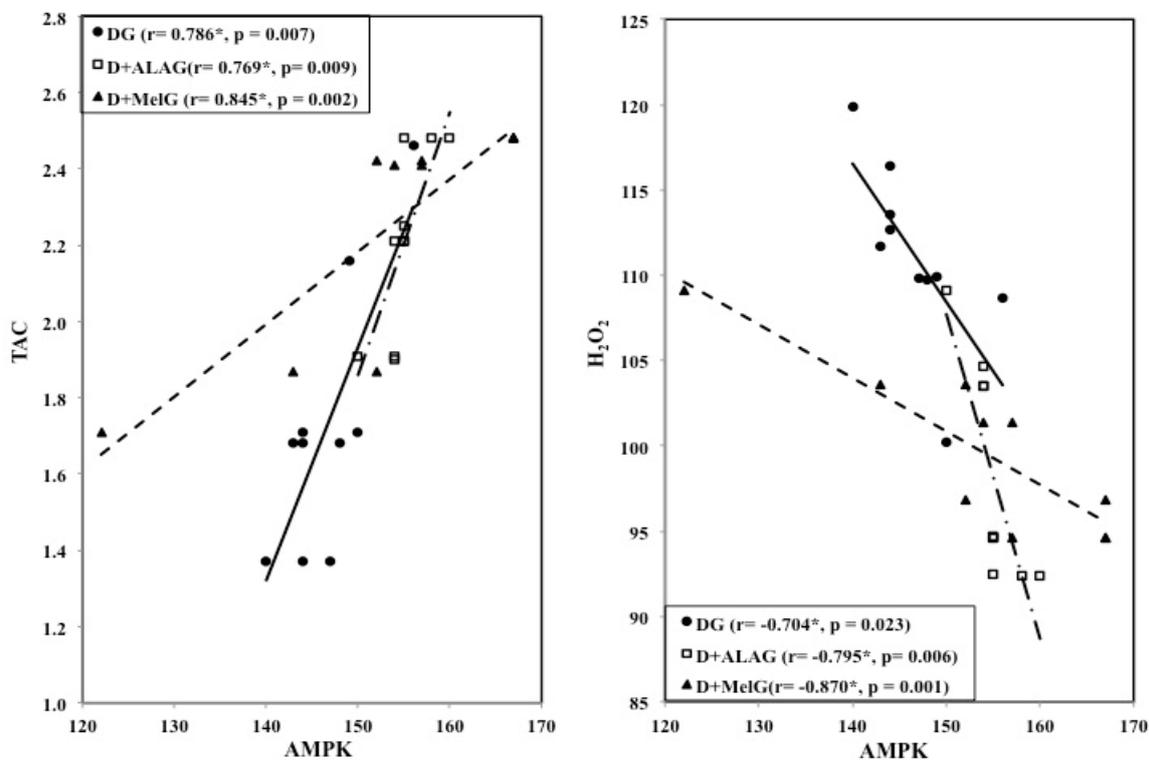


Figure 2; Correlation between AMPK activity and Oxidative stress in the cardiomyocytes. **a;** AMPK is positively correlated to the TAC in DG ($r = 0.786^*$, $p = 0.007$), D+ALAG ($r = 0.769^*$, $p = 0.009$) and D+MelG ($r = 0.845^*$, $p = 0.002$). **b;** AMPK is negatively correlated to the H₂O₂ levels in DG ($r = -0.704^*$, $p = 0.023$), D+ALAG ($r = -0.795^*$, $p = 0.006$) and D+MelG ($r = -0.870^*$, $p = 0.001$). AMPK; AMP-activated protein kinase, H₂O₂; Hydrogen peroxide, TAC; Total Antioxidant Capacity.

8. Effect of melatonin and ALA on cardiac tissues examined by histologic sections

As shown in fig. 3, normal cardiac myocytes were arranged longitudinally with vesicular oval central nuclei (fig.3A). In DCM group, cardiac myocytes showed variable degrees of disorganization and cytoplasmic degeneration with wide spaces

between cardiac muscle fibers (fig.3B). Treatment with melatonin and ALA caused restoration of normal architecture of cardiac myocytes with the presence of some wide intercellular spaces (fig.3 C&D).

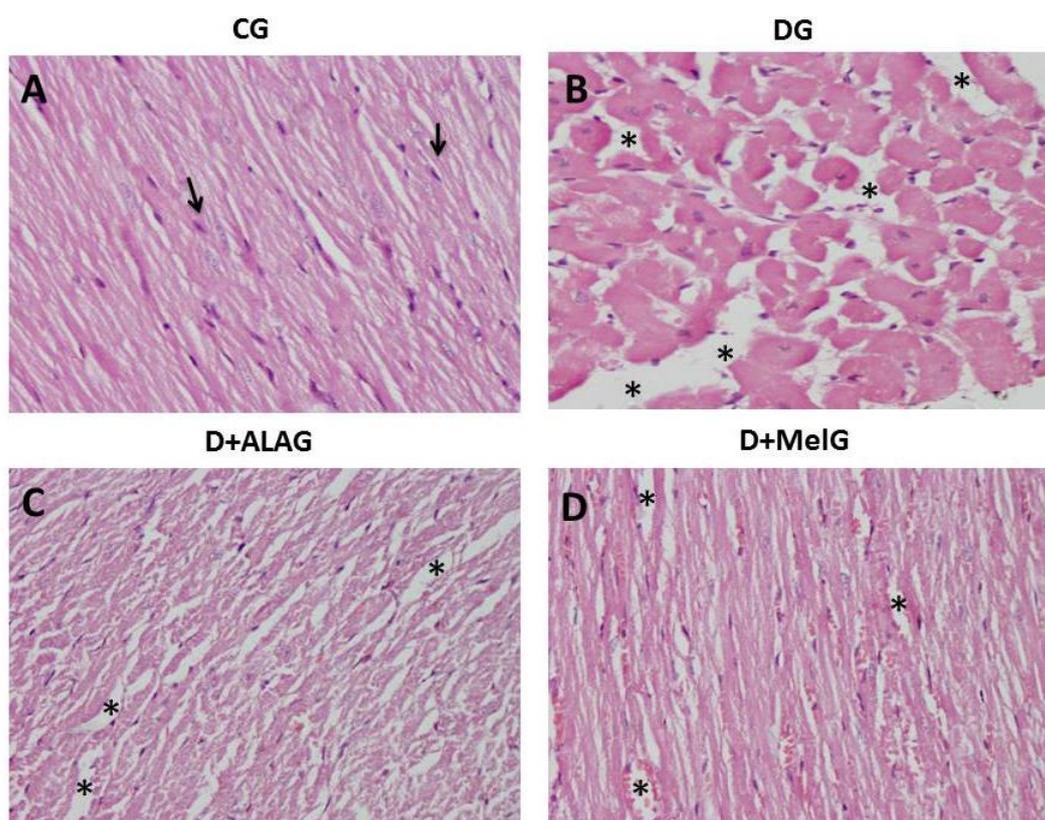


Figure 3: Representative photomicrographs of rat myocardium showing: in (A) normal cardiac myocytes arranged longitudinally with vesicular oval central nuclei (arrows) (normal control group). (B): transversally arranged cardiac myocytes with variable degrees of disorganization and cytoplasmic degeneration. Wide spaces were observed between cardiac muscle fibers (asterisks) (diabetic group). (C & D): longitudinally arranged cardiac myocytes with restoration of their normal architecture with still some wide intercellular spaces (asterisks). (C: Diabetic+ALA group and D: Diabetic+Mel group). Sections were stained with H&E and examined by light microscopy (magnification x400)

Discussion:

Hyperglycaemia, hyperlipidaemia and increased ROS (reactive oxygen species) are well known to be the cause of diabetic complications implicated in different body organs including the heart. They may induce alterations in transcription factors,

which result in changes in gene expression, myocardial substrate utilization, myocyte growth, endothelial function and myocardial compliance and thus may lead to cardiomyopathy. DCM was proved in this study by assessing the Ht/BW ratio that was significantly increased in diabetic rats compared to normal non-diabetic ones. In addition,

cardiac enzymes level was significantly increased in the former group, denoting the damage of cardiac myocytes. These results were in accordance with Wang et al (23), who considered the increase of Ht/BW ratio after the induction of diabetes, a marker for the development of diabetic cardiomyopathy. Melatonin and ALA treatment showed a positive effect on these parameters where the Ht/BW ratio was decreased in diabetic-treated groups compared to the untreated one. Also CK and LDH levels were significantly decreased. These data may denote the protective effect of melatonin and ALA against further damage to the cardiac myocytes. The exact mechanisms underlying the disease remain incompletely clear. Several pathological mechanisms responsible for DCM have been proposed(24). Although oxidative stress is widely considered as one of the major causes for the pathogenesis of the disease and probably contributes to the development, progression and complications of diabetes, but still the mechanism through which it causes cardiomyopathy needs to be investigated. In this study we hypothesized that ROS may mediate its damaging effects through a series of secondary transducers. One of which maybe the AMPK pathway alteration, being one of the central regulator of energy homeostasis, which coordinates metabolic pathways and thus balances nutrient supply with energy demand(25). Our results showed that AMPK activity was significantly decreased in hearts of diabetic rats. Although AMPK is known to be activated in response to a variety of conditions that deplete cellular energy levels, including nutrient starvation, especially glucose. The exact mechanism by which AMPK activity is decreased

in injured cardiac myocytes is unknown. But the decrease in AMPK activity was also reported by Xie et al(26), along with cardiac dysfunction and decreased cardiac autophagy in type I diabetic OVE26 mice. We did a correlation study between FBG level, lipids, oxidative stress, cardiac enzymes and AMPK activity to find an interpretation to the decreased AMPK activity in dysfunctional cardiac myocytes despite of the energy deprivation and oxidative stress that are natural activators to AMPK. AMPK activity was positively correlated to TAC and negatively correlated to H₂O₂ levels in the cardiac myocytes, suggesting that AMPK decreased activity in cardiomyopathy increased oxidative stress and thus contributes to more cardiac damage. This interpretation is more accepted rather than the oxidative stress being the cause of its deactivation. This was further supported by the negative correlation between CK, LDH and AMPK in which the decrease in AMPK activity is related to the increase of cardiac damage markers together with the increased oxidative stress. No correlation was found between AMPK activity, FBG level and lipids (data not shown). In this study, we demonstrate that treatment with melatonin and ALA suppressed the production of H₂O₂, enhanced the TAC in the myocardium. Thus, it is likely that both treatments elevate myocardial antioxidant activity and thereby inhibits the progression of cardiac myocytes damage and thus the expression of cardiac enzymes. Melatonin is known to be a potent endogenously occurring antioxidant that protects organisms from catastrophic oxidative stress (27). Likewise, ALA is an important natural antioxidant that is capable of scavenging a variety of ROS (28). In cardiac muscle, AMPK activation

stimulates glucose uptake, FA oxidation, and glycolysis (29). AMPK activity was significantly increased after melatonin and ALA treatment, which may also contribute to their cardioprotective effect. In accordance, Xie *et al* (26) concluded in his study that AMPK activation protects cardiac structure and function in the diabetic heart. Several drugs that have been used for the treatment of diabetes, such as thiazolidinediones (TZDs) and metformin, were found to exert some of their beneficial effects through the indirect activation of AMPK (30). In addition to pharmaceutical agents, numerous natural compounds and hormones can also activate AMPK. Even though being an apparently promising target for drug development, no direct AMPK activators have reached clinical use for the treatment of metabolic diseases. Perhaps Melatonin and ALA be of these therapeutic natural compounds, not only as AMPK activators but also as antioxidants, hypolipidemic and hypoglycemic drugs that even adds to their use in the prevention and/or treatment of diabetic complications. The hypolipidaemic effects of ALA and melatonin were shown with no significant difference between both except in the ameliorating effect of ALA on cholesterol level, which was significantly more than that of melatonin. The hypolipidemic effect of ALA was also reported in the study of Ford *et al.*(31) who studied the effects of an evening primrose oil supplement and an ALA supplement on a variety of lipid and hemostatic parameters in control and diabetic rats for 2 weeks. Supplementation with ALA at 300 mg/kg body weight caused a decrease in plasma triglyceride concentrations in diabetic rats. The same finding was reported by Amom *et al* (32) but in atherosclerotic rat model. The effect of

melatonin on lipid profile was also supported by Agil *et al* (33) where their results showed that melatonin raised high-density-lipoprotein (HDL) and significantly reduced low-density-lipoprotein (LDL) cholesterol in diabetic fatty rats but had no effect of total cholesterol level. ALA showed a hypoglycemic effect in this study, as it significantly decreased the FBG level in diabetic-ALA treated group in comparison to the untreated and the melatonin-treated diabetic ones. The blood glucose reducing effect was also shown by Ansar *et al* (34) who examined the effects of ALA treatment over a period of 2 months on FBG, insulin resistance, and glutathione peroxidase activity in type 2 diabetes patients, results showed a significant decrease in FBG in ALA-treated group. On the other hand melatonin did not affect FBG level in the current study. From another side, DCM was observed also in histological cardiac sections which showed wide spaces between cardiac muscle fibers with disorganized cardiac myocytes and cytoplasmic degeneration. Similar findings were reported by Dyntar *et al* (35) who showed that STZ-induced diabetes led to a significant increase in cardiac cell apoptosis and loss of intercellular contacts. While *in vitro*, prolonged exposure of cultured adult cardiac myocytes to high glucose concentrations reduced significantly myofibrillar formation (35). Treatment of diabetic rats with ALA and melatonin restored the normal architecture of cardiac myocytes with the presence of some wide intercellular spaces. Treatment with ALA has an anti-fibrogenic effect on diabetic hearts by reducing collagen accumulation which is considered as major risk factor for the progression of DCM (36). Also ALA treatment was reported to

increase the concentration of intracellular Ca^{+2} and the overexpression of sarcoplasmic reticulum Ca^{+2} . Both can protect hearts from severe contractile dysfunction (37,38). From another side, the beneficial effect of melatonin on cardiac muscle structure may be related to its ability to prevent apoptosis by modulating apoptosis regulatory proteins. Besides, melatonin was effective in the protection of cardiac myocytes membrane integrity (39). In conclusion, ALA was superior to melatonin in exerting a hypoglycemic and cholesterol lowering effect. These differences were partially reflected on their protective effect against DCM as there was a significant difference between the two treated groups regarding the reduction of Ht/BW ratio but not in the cardiac enzymes after 12 weeks treatment. May be longer treatment would have changed this parameter and thus supporting the ALA superiority in cardioprotection.

CONCLUSION

These results show that melatonin and ALA protect against the progression of diabetes mellitus-induced cardiac dysfunction by restoration of normal architecture of cardiac muscle fibers and attenuation of myocardial oxidative stress. This attenuation is probably not only through their hypolipidemic and antioxidant properties but also through the activation of AMPK which further leads to decrease in oxidative stress encountered in cardiac myocytes. ALA was only superior to melatonin in exerting a hypoglycemic, cholesterol-lowering effect, and thus showed more reduction in the Ht/BW ratio than that shown in melatonin-treated group, giving more protection against cardiac myocytes damage

than that offered by melatonin treatment.

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