Comparative Protective and Therapeutic Effects of Folic Acid on Cardiac Electrical and Structural Changes in a Rat Model of Diabetic-Cardiomyopathy

Original Article

Nanees Fouad EL-Malkey[1], Mariam Nasr Michael[1], Maher Nageb Ibrahim[1], Rania Said Moawad[2], Maha M. Ahmed Abdul Rahman[2] and Ebtesam Mohamed Ebrahim[1]

[1]Department of Medical Physiology, [2]Department of Human Anatomy and Embryology, Faculty of medicine, Zagazig University, Egypt

ABSTRACT

Introduction: Diabetic cardiomyopathy (DCM) is a cardiac disorder that could progress to heart failure. There is conflicting data about the role of folic acid supplementation in treatment DCM.

Aim of the Work: This study was performed to compare between the protective and therapeutic effects of folic acid on ECG and structural changes of DCM rat model.

Materials and Methods: Sixty rats were divided as follows: Normal control groups (Ia and Ib), DCM groups (IIa and IIb): Fed high fat diet for 4 weeks then injected with streptozotocin (25 mg/kg/ i.p), and folic acid treated groups (IIIa and IIIb): rats received 5 mg/kg/day orally for 8 weeks from first day of confirmation of diabetes in rats in group (IIIa), and from the end of 7th week after confirmation of diabetes in rats in group (IIIb). ECG, blood pressure, lactate dehydrogenase, Creatine kinase-MB, soluble receptor of advanced glycation end products (sRAGE), oxidative stress (OS) and inflammatory markers were assessed. Histopathological analysis of left ventricular tissue using Hematoxylin & eosin, Masson's Trichrome stains, and immunohistochemical analysis of Caspase3 and iNOS expression were performed.

Results: A significant improvement in R wave amplitude, ST segment deviation and a reduction in sRAGE in folic acid protective not therapeutic group were found when compared to untreated DCM group. Moreover, cardiac enzymes, histopathological changes, collagen fiber deposition, oxidative stress (OS), inflammation, and apoptosis significantly improved in both folic acid treatment groups when compared to untreated DCM groups.

Conclusion: The protective role of folic acid on cardiac injury in DCM rat model was more effective than the therapeutic ones that could be attributed to better glycemic, OS control and amelioration of AGE/RAGE signaling and improvement in cardiac structural changes.

Received: 10 May 2021, **Accepted:** 23 July 2021

Key Words: Apoptosis, cardiomyopathy, diabetes mellitus, folic acid, oxidative stress.

Corresponding Author: Rania Said Moawad, MD, Department of Human Anatomy and Embryology, Faculty of medicine, Zagazig University, Egypt, **Tel.**: +20 10 6000 3278, **E-mail:** karimwaleed62@gmail.com

ISSN: 1110-0559, Vol. 45, No. 4

INTRODUCTION

Diabetic cardiomyopathy (DCM) is a disorder characterized by myocardial structural and functional changes in diabetic patients with other diabetic comorbidities that leads to heart failure[1]. The pathophysiological links between diabetes and cardiomyopathy are multifactorial including direct effects of abnormal myocardial metabolism, even without atherosclerotic lesion or myocardial ischemia, including abnormal calcium signaling, disturbed glucose/fatty acid metabolism, inflammation, and insulin resistance (IR) that lead to fibrosis and stiffness of the myocardium[2], direct toxic effect on cardiomyocytes, endothelial dysfunction with thinning of vascular endothelium, weakening of intercellular junctions with increased permeability , altered protein synthesis and ultimately causes remodeling[3]. In addition, oxidative stress (OS) and reactive oxygen species (ROS) accumulation within cardiomyocytes results in cellular injury, increased incidence of apoptosis in the cardiomyocytes and promotes the development and progression of diabetic complications by activation of the mitochondrial pathway, cell death, cardiac diastolic and systolic dysfunction, and heart failure[1,4].

This multifactorial pathogenesis makes most of the current antioxidant therapeutic agents fail to improve the prognosis and outcome of this condition[5].

Notably, end products of advanced glycation (AGEs) are potent toxic molecules when bind to their receptor (RAGE), induce inflammation, OS, and cell death contributing to organ damage. They are also implicated in the induction of diabetic comorbidities such as cardiovascular disorders[6].

However, soluble form of RAGE (sRAGE) is a competitive receptor which binds to AGEs, inhibiting

 DOI: 10.21608/ejh.2021.75755.1479

them from binding to RAGE and preventing their pro-inflammatory cascade[7]. It has shown that sRAGE is interconnected to the cardiovascular diseases (CVD) in type1 and type 2 diabetes, however, its role in CVD is still argumentative. It is suggested that sRAGE may be considered as a monitor of CVD risk to assess the potentiality of interventions in view of regulating sRAGE[8].

Folic acid is a water-soluble vitamin that cannot be synthesized de novo and Humans rely on dietary intake to obtain sufficient levels of it[9]. Some clinical and experimental research revealed potential benefits of the use of folic acid in the treatment of cardiovascular pathology[10]. Folic acid could be implicated in reducing OS, and improving endothelial function[11]; however, it failed to reduce cardiac lactate dehydrogenase (LDH) as well as mitochondrial and cytosolic malondialdehyde (MDA) activity in the study of Mutavdzin *et al*.[12]. Additionally, Nkemjika *et al*.[13] found that Folic acid supplementation could not affect the incidence of major cardiovascular events, stroke, myocardial infarction, or mortality.

Mutavdzin *et al*.[12] suggested that drugs with anti-inflammatory, anti-OS and anti-diabetic properties could be more promising, as well as the identification of more specific markers, will lead to the demonstration of more unique strategies for diabetic cardiac complications management. Consequently, the aim of the present work is to demonstrate and compare the protective and therapeutic effects of folic acid administration on cardiac electrical, structural, and apoptotic changes, and to evaluate its effect on serum sRAGE in a trial to explore its possible mechanism/s in a rat model of diabetic cardiomyopathy.

MATERIAL AND METHODS

Animals

Total numbers of 60 healthy, adult male albino rats of local strains (170-200 g), were obtained from the Animals house, Faculty of Veterinary Medicine, Zagazig University. The animals were acclimatized to the animal house conditions for 2 weeks before the experiments were going on, kept under hygienic conditions in steel wire cages (4 rats/ cage). All animals had free access to food and water and kept at a comfortable temperature (24±2). The experimental protocol of the current study was approved by the "Institutional Animal Care and Use Committee of Zagazig University" (ZU-IACUC, Approval number: ZU-IACUC/3/F/142/2019).

Experimental design

Animals were randomly divided into three main equal groups as following:

Group I (control group; n= 20): That was subdivided equally into: Group Ia and Group Ib in which rats were fed normal chow diet all through the experimental periods (18% of energy derived from proteins, 5% from fat, and 77% from carbohydrates)[14]. At the beginning of 5th week animals in both groups were treated with intraperitoneal citrate buffer solution coinciding with the same dose and time in groups (IIa & IIb), then rats were treated with physiological saline gavage coinciding with the beginning of folic acid supplementation in group (IIIa & IIIb); respectively. Then, rats in Group Ia were let for 8 weeks and those in Group Ib were let for 15 weeks before scarification.

Group II (vehicle-treated DCM; n=20) which was subdivided into 2 equal subgroups: Group IIa and Group IIb: Rats were fed a high-fat diet (15.6% protein, 57.5% fat, 26.9% carbohydrate, Faculty of Agriculture, Zagazig University) all through the experimental periods. Then, at the beginning of 5th week animals were treated with 25 mg/kg streptozotocin intraperitoneally (Sigma chemical-Aldrich, St Louis, MO) diluted into precooled citrate buffer solution (1ml per 30 mg STZ at pH 4.5)[15]. Blood glucose was monitored weekly via tail-tip blood samples; rats with persistent blood glucose levels higher than 250 mg/dl after STZ administration were considered diabetic rats[16] and rats were treated with oral saline for 8 weeks, from confirmation of diabetes in rats in group (IIa), and from the end 7th week in group (IIb).

The period of the study protocol was following that of Patel & Goyal[17] who demonstrated that 8 weeks of experimentally induced type-2 DM could produce diabetic cardiomyopathy in rats.

Group III (DCM Treated with folic acid groups, n=20) that was subdivided into two equal subgroups: Group IIIa (folic acid -Protected group) and Group IIIb (folic acid -therapeutic group). Rats administered folic acid (5mg/kg b.w. /day) (MEPACO-MEDIFOOD, Enshas El Raml- sharkia-Egypt) dissolved in physiological saline by gavage (1.5 mL/rat) for 8 weeks[12], from first day of confirmation of diabetes in rats in group (IIIa), and from the end of 7th week after confirmation of diabetes in rats in group (IIIb)[10].

The chosen dose of Folic acid in the current work was dependent on the fact that Folic acid in doses of 5–20 mg/kg/ day could improve vascular dysfunction in chronic cardiac conditions[18]. In this study, 5 mg/kg/day was chosen to demonstrate the effect of the smallest dose in a trial to diminish unnecessary use of higher doses, which may have unwanted side effects.

Methods of the Study

1. Blood pressure measurement

Values of systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP) were obtained and recorded for each rat at the end of each experimental period of different study groups 8 weeks after the start of folic acid supplementation, using the noninvasive rat tail-cuff device "(NARCO, Biosystem, Inc., Huston, Texas).

2. Electrocardiography (ECG)

after 8–10 h overnight fasting, rats were anesthetized by urethane (1.5 g/kg i.p)[19], laid on the rodent surgical

table. Fifteen minutes after anesthesia, needle electrodes were inserted under the skin. Three-lead bipolar ECG is used. Positive, negative, and reference ECG electrodes were placed on the left foreleg, right foreleg, and left thigh; respectively[20].

The ECG apparatus (PowerLab4/20 (data acquisition system), AD Instruments Pty Ltd, Australia) was calibrated at 1mV/ 2 cm with speed of 5cm/ sec. Then, the results were automatically calculated based on the calibration value. R-R interval, ST-segment, R-wave amplitude, QT interval and heart rate were calculated from ECG recordings. ECG was recorded weekly for regular monitoring of cardiac electrical activity disturbance although the experiment.

3. Sampling of blood

Venous blood was collected by decapitation under anesthesia, in dry clean tubes, then left to clot for thirty minutes. Sera were separated by centrifugation for fifteen minutes, then stored frozen at -20˚C for further estimation of:

I. Serum glucose level enzymatically according to Finley and Tietz *et al*.[21], insulin levels using rat ELIZA kits " BioSource Europe S.A.-Rue de l'Industrie, 8-B- 1400 Nivelles-Belgium " following the instruction of Temple *et al*.[22] and homeostasis model assessment of insulin resistance (HOMA-IR) was Calculated according to the formula of Sun *et al*.[23] as following: HOMA-IR = insulin (µU/ml) × glucose (mg/dl) / 405.

II. Cardiac enzymes: Lactate dehydrogenase (LDH) and Creatine kinase–MB (CK-MB) were measured spectrophotometrically using the corresponding rat kits "Bio diagnostic- Egypt" following the method described by Moss *et al*.[24].

III. Serum malondialdehyde (MDA) enzyme assay by a spectrophotometer according Ohkawa *et al*.[25], serum superoxide dismutase (SOD) activity according to Kakkar *et al*.[26] by the corresponding rat kits "Sigma chemical- Aldrich, St Louis, MO".

IV. C-reactive protein (CRP) using immuno-enzymometric assay kits of rats "LifeSpan Bioscience, USA. Cat: LS-F26039- 1" according to Ridker *et al*.[27].

V. sRAGE level following Falcone *et al*.[28] instruction by ELISA kits for rats "Shanghai SunRed Biotechnology Co., Ltd".

Histopathological examination of cardiac tissue

I. Tissue Samples Collection and Processing

All animals were sacrificed at the expected time of the end of the experiment for each group. The collected heart tissues from left ventricle, were cleaned by saline, dried, and cut. Left ventricles were dissected, then fixed in 10% neutral buffered formalin solution. The tissues washed in tap water, after fixation, dehydrated in an ascended series of alcohols, and embedded in paraffin. 5-µm-thick sections were obtained from all specimens and stained with hematoxylin and eosin (H&E) for general histological examination and Masson's trichrome (MT) for collagen fibers[29]. After complete preparation, images were analyzed under an optical/light microscope with a digital camera attached at magnifications of 20 to 40×10 by an experienced investigator anatomist in a blind fashion.

II. Immunohistochemistry analysis:

Studying of Caspase 3 and Inducible nitric oxide synthase (iNOS) immune expression in the paraffin sections was applied as explained by Laurentino *et al*.[30] and Heger *et al*.[31], respectively. These methods were based on blocking of the unspecific binding by 10% goat serum. Then, either the specific anti-Caspase 3 or anti-iNOS antibodies were used to detect cellular content of Caspase 3 and iNOS. The sections were finally counter stained with hematoxylin and examined by a light microscope. Positive reaction was indicated by brown color.

All stained slides were examined by light microscopy (Leica ICC50W) in the Image analysis unit of the Department of Anatomy and Embryology, Faculty of Medicine, Zagazig University.

III. Morphometric analyses

An image analyzer (Image J software plugin) was used for the morphometric analysis, in the Anatomy and Embryology Department, Faculty of Medicine, Zagazig University.

Morphometric analyses were done to measure the area percentage of collagen fibers and area percentage of positive caspase 3 and iNOS immune stained cells by capturing the scattered representative fields in microscopic images at 400× magnification, and the percentage (%) areas stained with green color (positive areas) in Masson's trichrome stained slides and the percentage (%) areas stained with brown color (positive areas) in Caspase 3 and iNOS stained slides, were calculated in accordance with the method of Varghese *et al*[32].

The images were split into red, green, and blue stacks (RGB), and then the red stack was adjusted to a threshold to mark it with a binary mask. Then the percent area relative to the field was calculated at the objective lens of 40 X.

Statistical Analysis

Continuous variables were represented by the mean ± SD as the data showed normal distributions (parametric). The Kolmogorov-Smirnov test was used for checking the normality. One-way ANOVA was used to test the significant differences between groups. Multiple comparisons between groups of the same study duration were performed by Post hoc Tukey's test. However, the comparison between both folic acid treatment groups was done by an independent T test. At $P < 0.05$, the differences were considered significant. All statistical comparisons were two-tailed.

All statistical analysis was done using Graph pad Prism software, version 5.0 (Graph Pad Software, San Diego, CA, USA).

RESULTS

Effect of folic acid treatment on serum level of glucose metabolic parameters

This study showed a significant increase in glucose (p<0.001), and HOMA-IR (p<0.001), while insulin level was significantly decreased (p<0.001, p<0.01, p<0.001) in DCM groups (IIa, IIb) and Folic acid treatment groups (IIIa, IIIb) when compared with those in control groups (Ia, Ib; respectively). Additionally, there was significantly decreased glucose (p<0.001) and HOMA-IR (p<0.001), together with a significantly increased insulin level (p<0.001) in Folic acid treatment groups (IIIa, IIIb) in comparison to DCM untreated groups (IIa, IIb; respectively) (Table 1)

Regarding the comparison between the protective and therapeutic effects of folic acid treatment on gluco-metabolic parameters in group (IIIa) and (IIIb), a significantly increased glucose (p<0.001), HOMA-IR (p<0.01) associated with a significantly decreased serum insulin were observed in folic acid therapeutic group (IIIb) when compared with folic acid protective group (IIIa) (Table 1)

Effect of folic acid treatment on ECG Changes

PR interval showed a significant shortening in DCM group (IIa) (p<0.001), folic acid protective group (IIIa) (p<0.001) compared with control group (Ia) and in folic acid therapeutic group (IIIb) (p<0.001) compared to control (Ib). However, the PR interval was insignificantly changed in group (IIb) compared to control (Ib) (p>0.05).

Besides, a significant decrease in QT (p<0.001) intervals, R wave amplitude (p<0.001, p<0.001, p<0.05, p <0.001) which was associated with a significant increase in ST segment deviation (p<0.001) were present in DCM untreated groups (IIa, IIb) and folic acid treatment groups (IIIa, IIIb) when compared to those in control groups (Ia, Ib; respectively) (Figures 1,2).

Furthermore, a significant higher R wave amplitude (p<0.05) together with a significant less ST segment deviation (p<0.05) in folic acid protective group (IIIa) than those of untreated DCM group (IIa). However, no significant change was found between groups regarding PR and QT intervals (p>0.05) (Figures 1,2). In addition, PR interval was significantly shorter (p<0.001), associated with insignificant change in R wave amplitude, QT interval, and ST segment in folic acid therapeutic group (IIIb) (p>0.05) when compared with untreated DCM group (IIb) (p>0.05) (Figures 1,2).

Comparing folic acid protective and therapeutic groups showed a significant increase in R wave (p<0.001) and PR interval (p<0.001) in the protective group (IIIa) when compared to the therapeutic group (IIIb). However, insignificant change was found between groups regarding QT interval and ST segment (p>0.05) (Figure 3).

Effect of folic acid treatment on serum level of cardiac enzymes

There was a significant increase in LDH and CK-MB in untreated DCM groups (IIa and IIb) (p<0.001) and folic acid treatment groups (IIIa and IIIb) (p<0.001) when compared with those of their corresponding control group (Ia and Ib; respectively). Nevertheless, there was a significant decrease in LDH and CK-MB in folic acid treatment group (IIIa) (p<0.001) and (IIIb) (p<0.001) when compared with those of DCM group (IIa) and (IIb); respectively (Table 2). Regarding the comparison between the protective and therapeutic effects of folic acid treatment in group (IIIa) and (IIIb), serum LDH and CK-MB were significantly lower in group (IIIa) (p<0.001) than those in group (IIIb) (Table 2).

Effect of folic acid treatment on serum level of oxidative stress and inflammatory markers

As regard to sRAGE, its level showed a significant increase in untreated DCM groups (IIa, IIb) (p<0.001) and folic acid treatment groups (IIIa, IIIb) (p<0.001) when compared with that in the corresponding control group (Ia and Ib; respectively). However, it significantly decreased in folic acid protective group (IIIa) (p<0.05) when compared to that in untreated DCM group (IIa) (Table 2). However, there was an insignificant change between folic acid therapeutic group (IIIb) and untreated DCM group (IIb) (p>0.05) and folic acid protective group (IIIa) (p>0.05) (Table 2).

Interestingly, serum sRAGE exhibited a significant positive association with LDH (p< 0.001, 0.01), CK-MB (p< 0.001) and ST segment deviation (p< 0.01, 0.001), while it exhibited a significant negative association with R wave amplitude (p< 0.01), QT interval (p< 0.001, 0.01) in folic acid protective and therapeutic study groups, respectively. Additionally, a significant negative correlation was present between serum sRAGE and PR interval in folic acid protective study only (p<0.01) (Table 3).

Moreover, there was a significant increase in MDA (p<0.001) and SOD (p<0.001) in DCM group (IIa) (p<0.001) and folic acid protective group (IIIa) (p<0.05) when compared to control group (Ia). However, both parameters were significantly decreased in group (IIIa) (p<0.001) when compared to those in Group (IIa). Moreover, there was a significant increase in CRP in the DCM group (IIa) (p<0.01) when compared to that in the control group (Ia). However, there was a significant decrease in CRP in group (IIIa) (p<0.05) in comparison to group (IIa). While there was an insignificant change in CRP between group (IIIa) and group (Ia) (p>0.05) (Table2).

In addition, there was a significant increase in MDA that was associated with a significant decrease in SOD in DCM group (IIb) (p<0.001) and folic acid therapeutic group (IIIb) (p<0.001) in comparison to those in control

group (Ib). There was significantly lower MDA (p<0.001), concomitant with a significantly higher SOD in folic acid therapeutic group (IIIb) (p<0.05) than those of DCM group (IIb). However, there was a significant increase in CRP in DCM group (IIb) and folic acid therapeutic group (IIIb) (p<0.001, p<0.05) when compared to the control group (Ib). While there was a significant decrease in CRP in group (IIIb) (p<0.01) when compared to that in group (IIb) (Table 2).

Effect of folic acid treatment on blood pressure and HR

There was a significant increase in SBP (p<0.05, p<0.001), DBP (p<0.001), MAP (p<0.001) and HR (p<0.001) in untreated DCM groups (IIa, IIb) when compared to those in control groups (Ia, Ib; respectively), while, SBP (p<0.05, p<0.001), DBP (p<0.001) and MAP (p<0.001) in folic acid protective and therapeutic groups (IIIa, IIIb) were significantly lower than those in group (IIa, IIb; respectively), which was associated with an insignificant change in HR between groups. Additionally, there was a significant increase in SBP (p<0.001), MAB (p<0.05) and HR (p<0.001) in group (IIIb) when compared to group (Ib). However, there was an insignificant change in SBP, DBP, MAP between group (IIIa) and group (Ia). Moreover, regarding DBP in group (IIIb) and (Ib), there was an insignificant change between groups (p> 0.05) (Table 4).

Regarding the comparison between the protective and therapeutic effects of folic acid treatment on blood pressure in groups (IIIa) and (IIIb); respectively, , there was a significant increase in SBP (p<0.001), DBP (p<0.05), MAP (p<0.001) in Group IIIb when compared to those in Group IIIa, however, there was an insignificant change between both groups regarding HR (p>0.05) (Table 4).Interestingly, serum sRAGE showed a significant positive correlation with SBP (p< 0.01), DBP (p< 0.001, 0.01), MAP (r=0.63, p< 0.01, 0.001) and HR (0.01, 0.05) in folic acid protective and therapeutic study; respectively (Table 5).

Effect of folic acid treatment on cardiac tissue structure in Hx& E-stained cardiac sections

Histopathological analysis of the normal control groups (Ia and Ib) in the present work showed the characteristic histological structure of the cardiomyocytes. The myocytes appeared slender, branching, and anastomosing, with large central oval vesicular nuclei. Moreover, transverse cross-striations and intercalated disks were clearly observed in the cardiomyocytes (Figures 4a,b).

However, sections of untreated DCM groups (IIa, IIb) showed some histological affection in the form of wavy cardiomyocytes with apparent cellular exudate in between them. Moreover, some of the nuclei appeared irregular and deeply stained. Furthermore, vacuolation of the cytoplasm of some cardiac myocytes was detected. Disruption and fragmentation of cardiac myocytes was also seen, with dilated congested blood capillaries between them, and other cardiomyocytes appeared normal with central vesicular oval nuclei. Notably, the histopathological changes in group (IIb) were more marked and evident than those in group IIa (Figures 5,6).

While in folic acid protective and therapeutic groups (IIIa and IIIb), most cardiac muscle fibers achieved their normal appearance and arrangement, showing slender, branching, and anastomosing cardiac muscle fibers with central oval vesicular nuclei that was nearly like to the control group. Nevertheless, a few cardiomyocytes were with darkly stained nuclei and in some areas, there was separation of myocardial fibers with loss of their striations. Moreover, mild congestion of blood capillaries was detected in between the cardiomyocytes. However, the improvement in group (IIIa) was more than that of (IIIb), as more nuclei were still irregular, darkly stained, more muscle fibers showed loss of striations and more marked congested, dilated blood vessels were still observed in between cardiomyocytes in the later (Figures 7,8).

Effect of folic acid treatment on cardiac tissue collagen fibers deposition in Masson's Trichrome stained- cardiac sections

Cardiac sections obtained from the control groups (Ia, Ib) showed scanty collagen fibers between the cardiac myocytes (Figures 9a,d). However, in DCM groups (IIa, IIb), there were abundant collagen fibers between cardiac myocytes (Figures 9b,e) compared with the control groups with a significant increase in area% of collagen deposition. Interestingly, folic acid treatment groups (IIIa, IIIb) showed minimal collagen fibers between the cardiac myocytes (Figures 9c,f) compared with DCM groups, while area% of collagen deposition in group (IIIb) was significantly higher than that of group (IIIa) (Table 6).

Effect of folic acid on Immunohistochemical expression of caspase 3

There was no significant expression of caspase 3 in cardiac cells, in the control groups Ia and Ib (Figures 10a,d). In DCM groups IIa and IIb groups, there were significantly increased immune positive cells compared to the control groups (Figures 10b,e). However, folic acid protected group (IIIa) and therapeutic group (IIIb) showed significant decrease in immune positive cells compared to those of the groups (IIa) and (IIb), respectively (Figures 10c,f). But the expression was still significantly more than in the control groups (Ia) and (Ib). Moreover, immune positive cells expression in group (IIIb) was significantly more than that in group (IIIa) (Table 6).

Effect of folic acid on Immunohistochemical expression of iNOS

There was no significant expression of iNOS in cardiac cells, in the control groups Ia and Ib (Figures 11a,d). In DCM groups IIa and IIb groups, there were a significantly increased immune positive cells compared to the control groups (Figures 11b,e). However, folic acid protected group

(IIIa) and therapeutic group (IIIb) showed a significant decrease in immune positive cells compared to those of the groups (IIa) and (IIb), respectively (Figures 11c,f). The expression showed no significant difference between group IIIa and control group (Ia), but the expression in group IIIb was still significantly more than in the control groups (Ib). Moreover, immune positive cells expression in group (IIIb) was significantly more than that in group (IIIa) (Table 6).

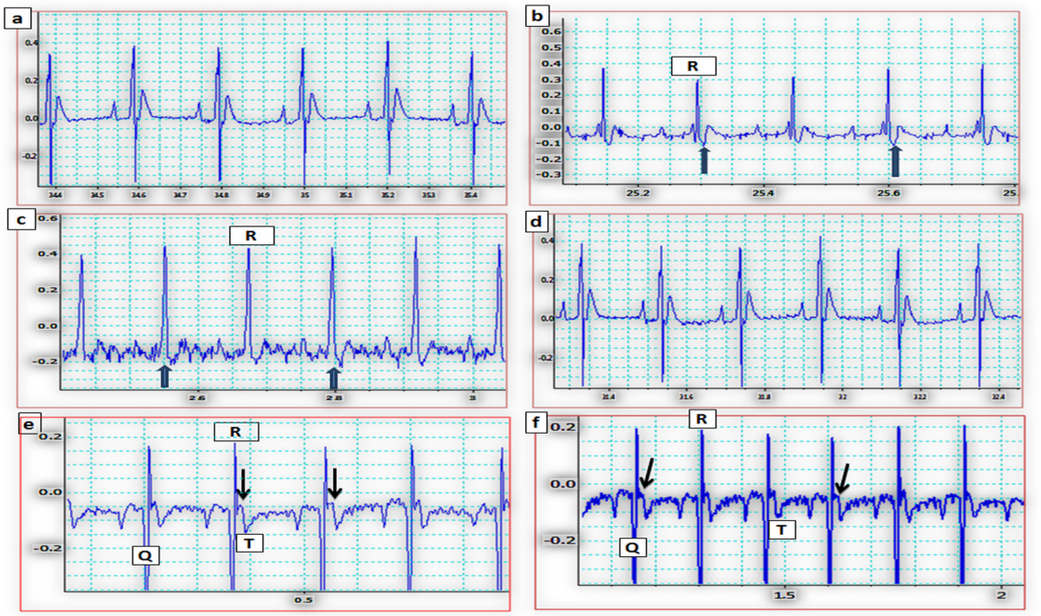

Fig 1: ECG tracing in different study groups showing: a & d) Normal ECG tracing in control groups (Ia &Ib), b) Lowered "R" wave amplitude (R), and depressed S-T segment (arrow) in DCM group (IIa), c) Improved "R" wave amplitude (R), with decreased ST segment depression (arrow) in folic acid protective group (IIIa), e) Pathological Q (Q), Low R wave amplitude (R), raised S-T segment (arrow), and inverted T wave (T) in DCM group (IIb), f) Pathological Q (Q), Low R wave amplitude (R), raised S-T segment (arrow), and inverted T wave (T) in folic acid therapeutic group (IIIb).

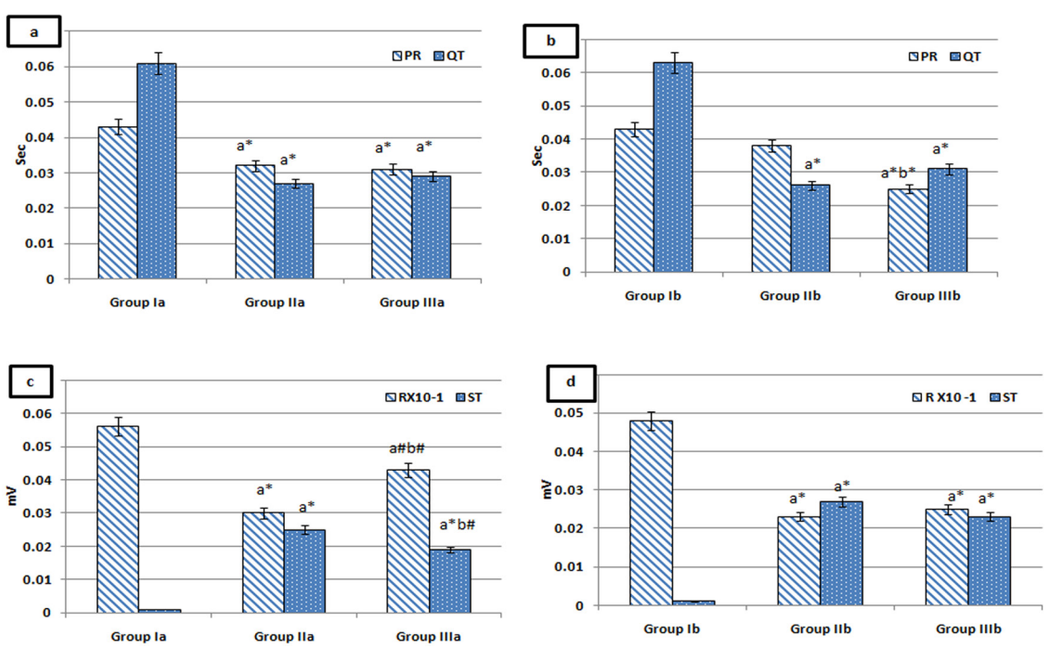

Fig. 2: Comparing means of electrical changes in all groups.
a: significant vs normal control, b: significant vs DCM group. *: $p<0.001$, #: $p<0.05$

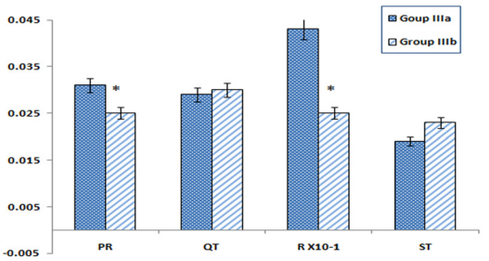

Fig. 3: Comparing means of electrical changes in folic acid treatment groups.
*: significant vs group IIIa

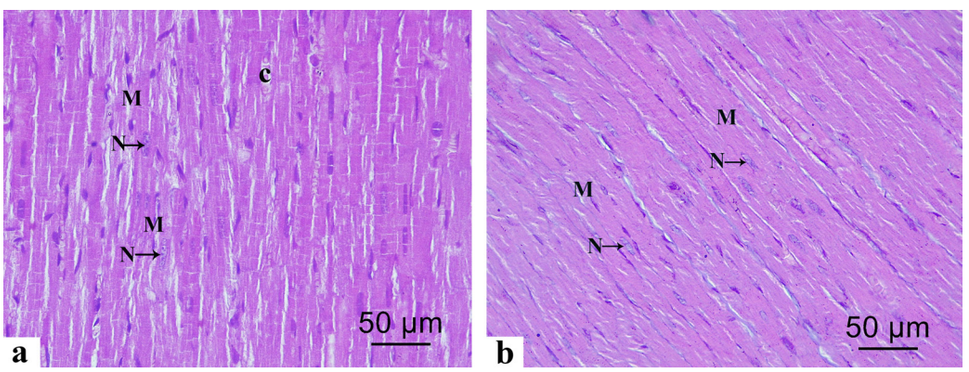

Fig. 4: Photomicrographs of L.S left ventricular muscles of an adult rat in Group Ia (a) and Group I b (b) showing slender, branching, and anastomosing myocytes, with transverse cross-striations and intercalated disks (M), with large central oval vesicular nuclei (N). Blood capillary is also seen in between myocytes (c). (H&E X 400, scale bar = 50µm).

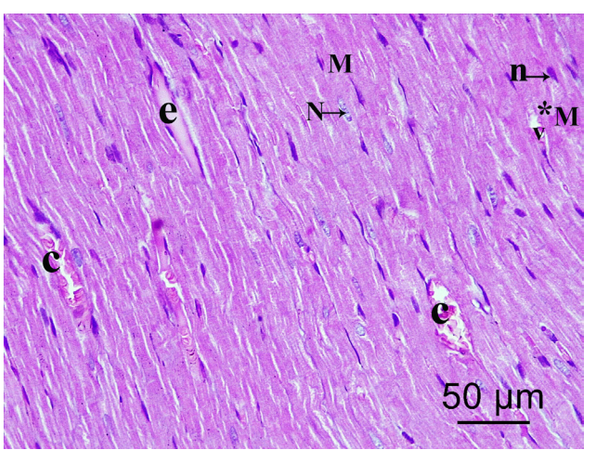

Fig. 5: A photomicrograph of L.S left ventricular muscles of an adult rat in Group IIa showing disruption and fragmentation of cardiac myocytes with loss of their striations (*M), and cellular exudate (e) is apparent in between the cardiac myocytes. Some nuclei of myocytes appear irregular and deeply stained (n). Also, vacuolation of the cytoplasm appears (v). Dilated congested blood capillaries (c) are also detected between cardiac myocytes. Other cardiomyocytes appear with normal striations (M)and with central vesicular oval nuclei (N). (H&E X 400, scale bar = 50µm).

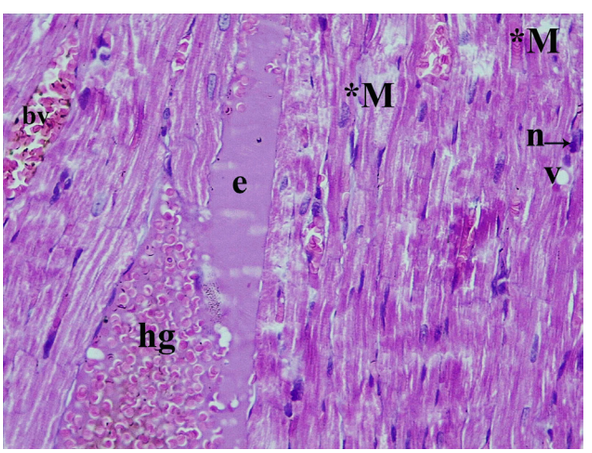

Fig. 6: A photomicrograph of L.S left ventricular muscles of an adult rat in Group IIb showing that there is disruption and fragmentation of cardiac myocytes with wide spaces in between and loss of their striations (*M). Most nuclei of cardiomyocytes are irregular, darkly stained nuclei (n) with vacuolated cytoplasm (v). Cellular exudate (e) is also observed in between the cardiomyocytes. Dilated congested blood vessels (bv) with hemorrhage (hg) are also seen in between the cardiomyocytes. (H&E X 400, scale bar =50 µm).

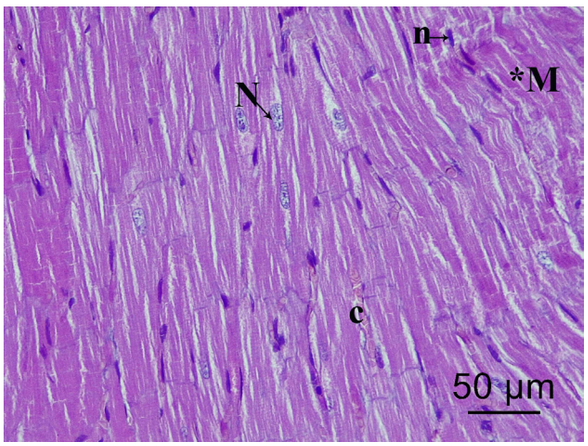

Fig. 7: A photomicrograph of L.S left ventricular muscles of an adult rat in Group IIIa showing most of the cardiac muscle fibers achieve their normal appearance and arrangement as they become slender, branching, and anastomosing with central oval vesicular nuclei (N). A few cardiomyocytes exhibit irregular, darkly stained nuclei (n). Moreover, mild congestion of blood capillaries (c) is detected in between the cardiomyocytes. Disruption and fragmentation of cardiac myocytes with loss of their striations (*M) in some areas are also seen. (H&E X 400, scale bar = 50µm).

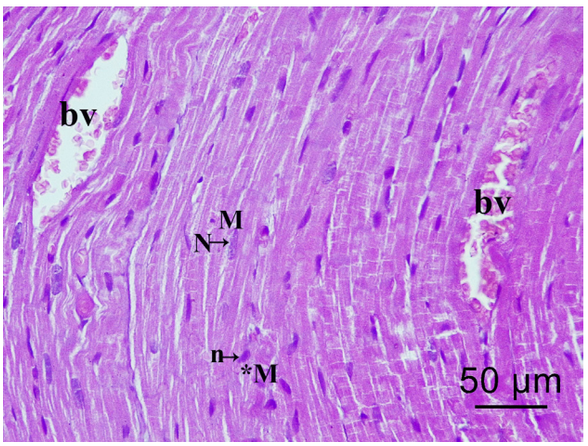

Fig. 8: A photomicrograph of L.S left ventricular muscles of an adult rat in Group IIIb showing that some myocytes appear slender, branching, and anastomosing (M), with large central oval vesicular nuclei (N) with longitudinal striations. Other muscle fibers show loss of longitudinal striations (*M) with irregular, darkly stained nuclei (n). There are marked congested, dilated blood vessels in between cardiomyocytes (bv) (H&E X 400, scale bar =50 µm).

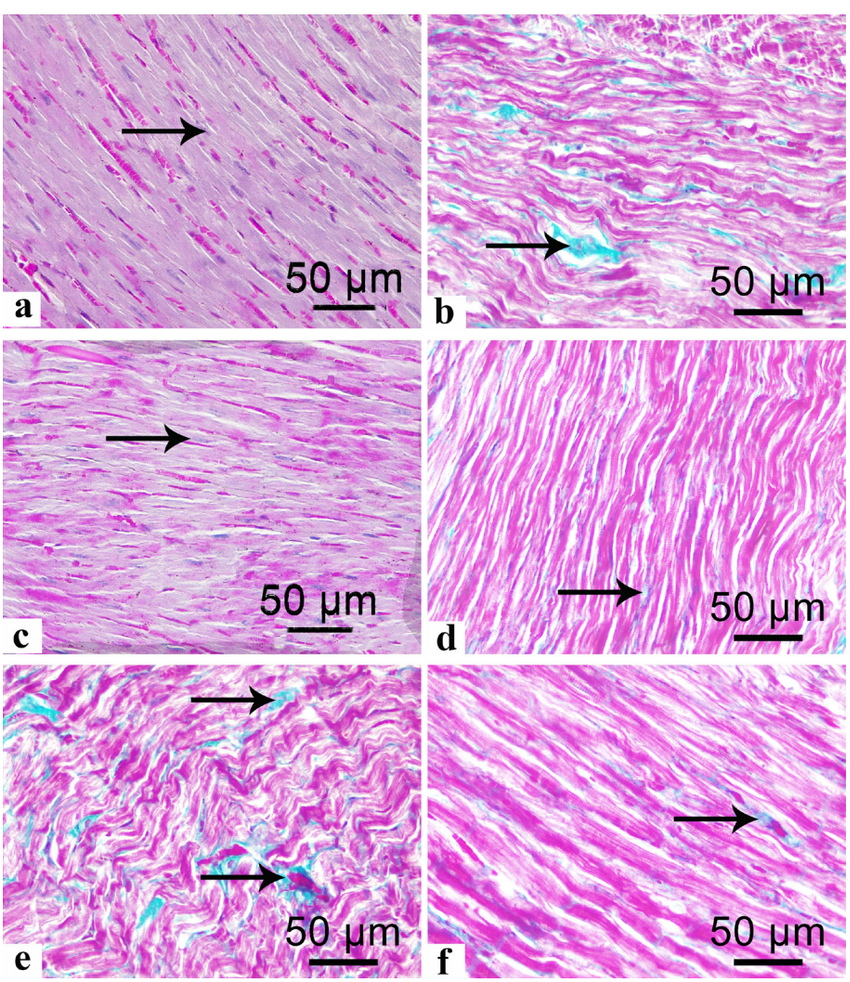

Fig. 9: A photomicrograph of collagen fiber deposition in L.S of left ventricular cardiac muscles of adult rats in different groups showing in a & d) Groups Ia and Ib, there are scanty collagen fibers between the cardiac myocytes. In b & e) Group IIa and IIb, there are abundant collagen fibers between cardiac myocytes. In c & f) Group IIIa and IIIb, there are minimal collagen fibers between the cardiac myocytes (MT × 400, scale bar =50 µm).

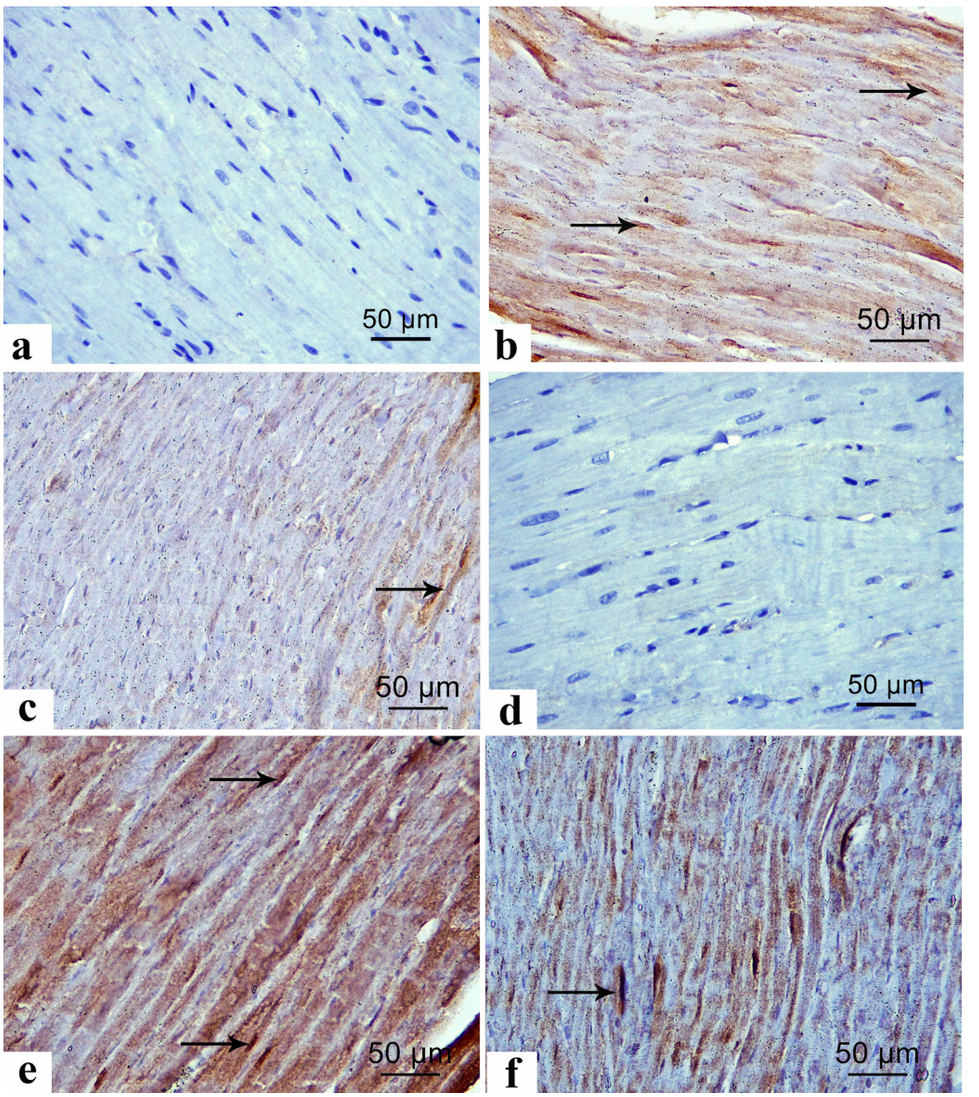

Fig. 10: A photomicrograph of caspase 3 immunohistochemical staining of cardiac muscles of different groups showing: in a & d) No expression of caspase 3 in cardiac cells in control groups (Ia and Ib; respectively), in b & e) An increase of caspase 3 positive cells expression (arrows) in untreated DCM groups (IIa and IIb; respectively) and in c & f) Minimal expression of caspase 3 positive cells (arrow) in folic acid treated groups IIIa & IIIb; respectively compared to groups (IIa) and (IIb) (Immunoperoxidase technique for caspase 3 X 400, scale bar =50 µm).

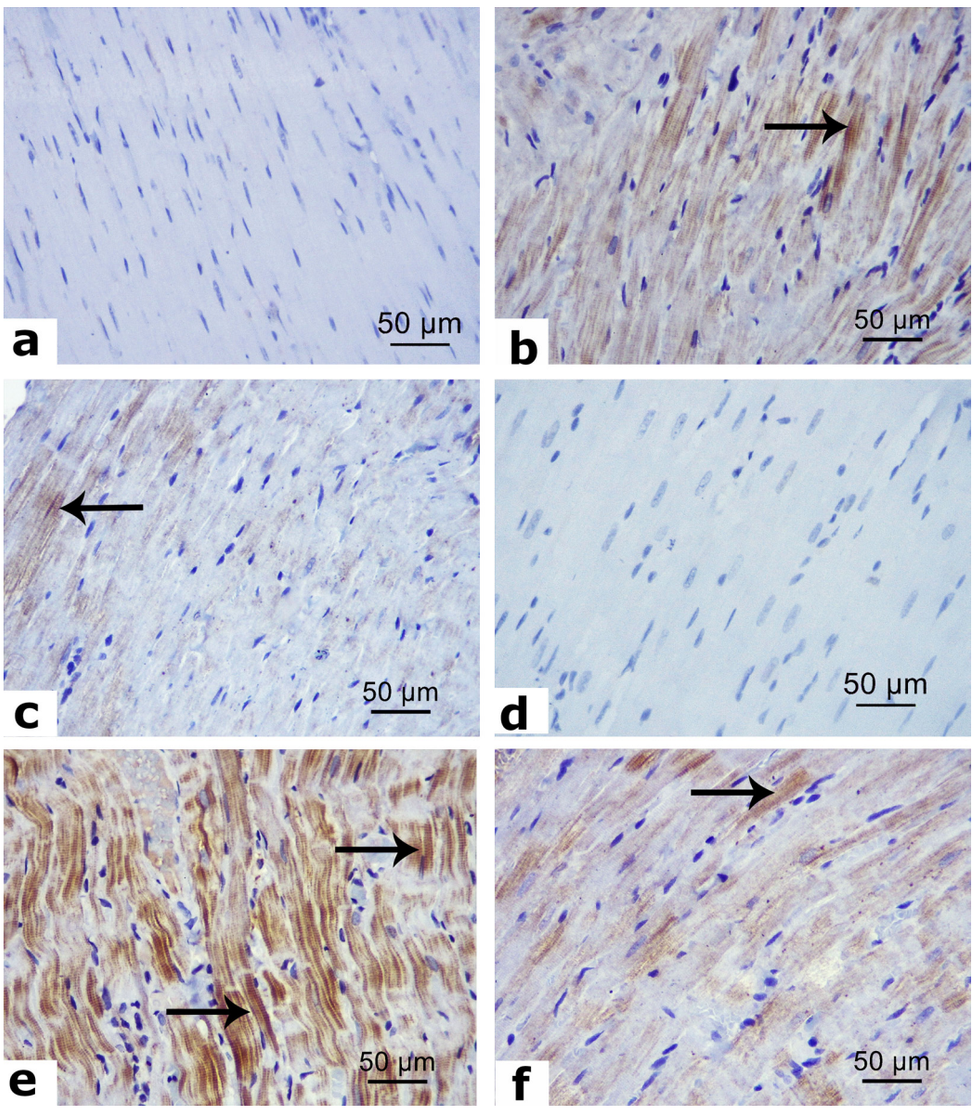

Fig. 11: A photomicrograph of iNOS immunohistochemical staining of cardiac muscles of different groups showing: in a & d) No expression of iNOS in cardiac cells in control groups (Ia and Ib; respectively), in b & e) An increase of iNOS positive cells expression (arrows) in untreated DCM groups (IIa and IIb; respectively) and in c & f) Minimal expression of iNOS positive cells (arrow) in folic acid treated groups IIIa & IIIb; respectively compared to groups (IIa) and (IIb) (Immunoperoxidase technique for iNOS X 400, scale bar =50 µm).

Table 1: Serum levels of gluco-metabolic parameters all groups

	Group (Ia)	Group (IIa)	Group (IIIa)	Group (Ib)	Group (IIb)	Group (IIIb)
Glucose (mg/dl)	92.6±17.5	480±44.6^{a*}	208± 21.1^{a*b*}	87.1±11.9	533±85.4^{c*}	333±41.1^{c*d*t*}
Insulin (µIU/ml)	26.2±1.2	18.5±1.9^{a*}	24± 0.5^{aSb*}	26.2±0.9	20.01±0.7^{c*}	22.8±0.7^{c*d*t*}
HOMA-IR	5.9±0.95	21.9±2.2^{a*}	12.4±1.3^{a*b*}	5.6±0.8	26.3±4^{c*}	18.7± 2.1^{c*d*t*}

a: significant vs group (Ia), b: significant vs group (IIa), c: significant vs group (Ib), d: significant vs group (IIb); t: significant independent sample t test between groups (IIIa & IIIb), *: $p<0.001$

Table 2: Serum level of cardiac enzymes, oxidative stress and inflammatory markers in all groups

	Group (Ia)	Group (IIa)	Group (IIIa)	Group (Ib)	Group (IIb)	Group (IIIb)
LDH (U/L)	364.5± 65.6	969.6±142.1 ^{a*}	601.8±50.2 ^{a*b*}	369.8± 63	1280.5±365.7 ^{a*}	785.4±50.4 ^{c*d*t*}
CK-MP (U/L)	2.6±0.8	16.8 ± 2.4 ^{a*}	6.6 ± 0.8 ^{a*b*}	1.03± 0.28	18.9± 1.7 ^{a*}	9.8± 0.96 ^{c*d*t*}
sRAGE(ng/mL)	5.3± 0.5	6.4± 0.3 ^{a*}	5.9± 0.1 $^{a*b\#}$	5.2± 0.5	6.2± 0.4 ^{c*}	5.8± 0.4 $^{c\#}$
MDA(nmol/mL)	9.0±0.8	17.1±0.5 ^{a*}	11.5±0.3 ^{a*b*}	9.4±0.8	16.9±0.4 ^{c*}	13.3±0.8 ^{c*d*t*}
SOD(U/mL)	24.4±0.6	28.5±0.5 ^{a*}	25.2±0.6 $^{a\#b*}$	25± 0.8	20.44± 0.67 ^{c*}	23.7± 01.08 $^{c*d\#}$
CRP(mg/dL)	1.8± 0.6	2.9±1.0 $^{a\$}$	2.0± 0.5 $^{b\#}$	1.3± 0.5	3.3±0.9 ^{c*}	2.2±0.6 $^{c\#d\$}$

a: significant vs group (Ia), b: significant vs group (IIa), c: significant vs group (Ib), d: significant vs group (IIb); t: significant independent sample t test between groups (IIIa & IIIb), *: p<0.001, #: p<0.05

Table 3: Correlation coefficient between sRAGE and cardiac parameters in all groups

	Folic acid protective study	Folic acid therapeutic study
LDH	+0.79*	+0.58$^{\$}$
CK	+0.73*	+0.69***
PR	-0.57$^{\$}$	-0.14 ^{NS}
R	-0.56$^{\$}$	-0.53$^{\$}$
ST	+0.75*	+0.63*
QT	-0.70*	-0.58$^{\$}$

*: p<0.001, $: p<0.01, NS: nonsignificant

Table 4: Blood pressure, heart rate in all groups

	Group (Ia)	Group (IIa)	Group (IIIa)	Group (Ib)	Group (IIb)	Group (IIIb)
SBP (mmgH)	123.8± 4.1	147.5± 4.6$^{a\#}$	122.5±9.2$^{b\#}$	126.5± 4.1	147.5± 4.9^{c*}	134.7± 3.2^{c*d*t*}
DBP (mmgH)	73.7± 6.3	97.8± 4.7^{a*}	76.5± 3.7^{b*}	78.2± 4.9	97.1± 5.1^{c*}	82± 4.9$^{d*t\#}$
MAP (mmgH)	90.4± 4.5	113.3± 4.6^{a*}	91.8± 3.7^{b*}	94.3± 3.7	114± 5.0^{c*}	99.6± 3.0$^{c\#d*t*}$
MAP (mmgH)	298.8± 4.7	367.1± 13.7^{a*}	361.2± 14.7^{a*}	301.5±505	370.5± 16.6^{c*}	377.5± 23.6^{c*}

a: significant vs group (Ia), b: significant vs group (IIa), c: significant vs group (Ib), d: significant vs group (IIb); t: significant independent sample t test between groups (IIIa & IIIb), *: p<0.001, #: p<0.05

Table 5: Correlation coefficient between sRAGE, blood pressure and HR

	Folic acid protective study	Folic acid therapeutic study
SBP	+0.51$^{\$}$	0.64*
DBP	+0.63*	0.58$^{\$}$
MAP	+0.63*	0.68*
HR	+0.76*	0.48*

*: p<0.001, $: p<0.01,

Table 6: Area percentage of collagen fibers, caspase3 and iNOS immune positive cells in ventricular tissue of all groups

	Group (Ia)	Group (IIa)	Group (IIIa)	Group (Ib)	Group (IIb)	Group (IIIb)
(%) of collagen fibers	0.51±0.19	3.82± 1.43^{a*}	1.03± 0.28^{a*b*}	0.36±0.19	6.61± 1.32^{c*}	3.09±0.88^{c*d*t*}
(%) caspase-3 immunoexpression	1.33±0.54	18.20± 1.69^{a*}	6.53±1.47a*b*	1.25±0.54	37.85±3.97^{c*}	18.88±4.08$^{c*d*t\$}$
(%) iNOS immunoexpression	0.69±0.35	10.14±1.89^{a*}	3.17± 1.44^{b*}	1.03±0.34	34.35±4.59^{c*}	12.41±4.74^{c*d*t*}

a: significant vs group (Ia), b: significant vs group (IIa), c: significant vs group (Ib), d: significant vs group (IIb); t: significant independent sample t test between groups (IIIa & IIIb), *: p<0.001, #: p<0.05

DISCUSSION

Type-2 DM was proven by the significant hyperglycemia, hyperinsulinemia and the increase in HOMA-IR in DCM untreated groups (IIa and IIb) in comparison with the corresponding control group (Ia and Ib; respectively), that was in accordance with the study of Guo *et al*.[33], who reported that injection of low-dose of STZ induces a gradual impairment of insulin secretion, which is similar to the natural progression course of type-2 DM, as STZ has a cytotoxic action via increasing the production of nitric oxide (NO) and damaging the nuclear DNA of pancreatic β-cells.

Regarding evidence of developing DCM, the recorded ECG tracings showed a significant deviation of "S-T" segment (depression and elevation, respectively), decrease in "R" wave amplitude, shortening of "PR" interval, and "QT" interval, and increase in HR when compared to the control groups (Ia and Ib; respectively). The present findings agreed with those of Sabourin *et al*.[34] who observed the inhibition of transient receptor potential ion channels (TRPC) could decrease the "R" wave amplitude, shorten the "PR" interval, and induce ventricular arrhythmias in diabetic hearts, indicating that TRPC channels may have a role in the regulation of the electrical conduction through the AV node, and the ventricles.

In addition, Vinik *et al*.[35] and Murtaza *et al*.[4] explained these ECG changes by dysregulation of the autonomic nervous system and the development of diabetic cardiovascular autonomic neuropathy (CAN) that could lead to a sympathetic over-activation, and modulation of the activity of the sinus node.

Furthermore, DCM model in the present study revealed an increase in serum cardiac enzymes (LDH and CK-MB) in comparison with the control groups. These data agreed with Upaganlawar and Balaraman[36].

LDH and CK-MB present primarily in cardiac myocytes. Elevated serum LDH activity is a trustable indicator of organ injury, infarction and/ or necrosis, especially those adversely affected by DM[36] via increased the production of reactive nitrogen and oxygen species which could result in tissue and organ damage[37]. In contrast, Saleem *et al*.[38] showed that the decrease in the levels of cardiac enzymes was a sign of the tissue injury due to lipid peroxidation.

This observation was supported by the histopathological changes of cardiac tissue in untreated DCM groups (IIa, IIb) in the present work as there was wavy cardiomyocytes with apparent cellular exudate in between them, irregular and deeply stained nuclei, vacuolation of the cytoplasm of some cardiac myocytes, disruption, and fragmentation of cardiac myocytes with dilated congested blood capillaries between them with a significant increase in area percentage of collagen fiber deposition between the cardiac tissues, which confirmed the damage in cardiac muscle. Notably, the histopathological changes in group (IIb) were more marked and evident than those in group (IIa). These findings were agreed with Thent *et al*.[39] and Wu *et al*.[40] who reported that in streptozotocin induced diabetic rats there were structural organization of cardiac tissues in the form of deformation of nuclei of cardiomyocytes and disarrangement or disordered cardiac myofibers and there was an increase in collagen fiber deposition between the cardiac tissue.

Immunohistochemical staining for caspase 3, an important indication of apoptotic cell death, in DCM groups (IIa, IIb) showed a significant increased immune positive cell compared to the control groups. These results agreed with that of Yu *et al*.[41] who reported that there was higher expression of Bax and caspase-3 but lowered expression of Bcl-2 in DM group compared to control group. Abdel-Hamid *et al*.[42] stated a significant increase in the caspase-3 expression in cardiomyocytes of the DCM group.

Soldani and Scovassi[43] reported that reactive oxygen species (ROS) has been shown to be an important apoptotic signal. This apoptotic pathway ultimately increases caspase-3 and caspase-7, which leads to the activation of poly (ADPribose) polymerase (PARP), a well-known substrate of caspase-3; this cleavage ultimately leads to the morphological and biochemical changes that are characteristic of apoptotic cells.

The interesting finding of the present study is the significant partial improvement in ECG changes as there was a significant increase in "R" wave amplitude associated with a decrease in "ST" segment deviation in folic acid protective group (IIIa). Additionally, there was a significant decrease in cardiac enzymes (LDH and CK-MB) in both folic acid-treated groups (IIIa and IIIb) when compared with DCM untreated groups (IIa and IIb; respectively). However, insignificant changes in "PR" and "QT" intervals were observed in comparison to untreated groups. These observations were in line with that observed by Stanhewicz and Kenney[44].

The previous results were alongside with improvement in cardiac tissue microscopic findings in folic acid protective and therapeutic groups (IIIa and IIIb), as there was narrower intercellular spaces between cardiac myocytes that appeared normal with central vesicular oval nuclei, despite the presence of some irregular and deeply stained nuclei, additionally, a significant decrease in the percentage area of collagen deposition however, the improvement in group (IIIa) was more than that of (IIIb). These findings agreed with that Stanhewicz and Kenney[44] and of Li *et al*[45] who revealed that folic acid supplementation decreased both enzymes' activities and corrected the cardiac structural abnormalities in diabetic and HFD-fed mice and markedly reduced the degree of collagen deposition and fibrosis. The previous authors proved that folic acid improved oxidative stress and myocardial fibrosis in the heart.

Interestingly, in the present work the immunohistochemical staining for caspase 3 showed a significant reduction in the immune positive cells in the rats treated with folic acid in comparison with the untreated ones that agreed with the findings of Shafikhani *et al*.[46]

who reported that folic acid was able effectively to inhibit endothelial cell apoptosis.

The anti-apoptotic effect of folic acid could be related to the antioxidant and anti-inflammatory properties of folic acid as the exposure of endothelial cells in *vitro* to high levels of glucose caused significant ROS formation in company with caspase-3 activation and apoptosis. Both the activation of caspase-3 and the induction of apoptotic cell death could be inhibited by addition of antioxidants in the cultures[47].

It is worth noticing that the results of the present study revealed a significant increase in sRAGE in untreated DCM groups (IIa and IIb) and folic acid treated groups (IIIa and IIIb) in comparison with the corresponding control groups. However, only in the protective group (IIIa), sRAGE significantly decreased when compared to DCM untreated group (IIa).

Furthermore, this study revealed a significant positive correlation between sRAGE and cardiac enzymes (LDH, CK-MB), also there was a significant positive correlation between sRAGE and SBP, DBP, MBP, HR and ST segment, but there was a significant negative correlation between sRAGE and PR interval, QT interval and R wave amplitude.

This was supported by Yamagishi *et al*.[48] who demonstrated that hyperglycemia contributes to the formation of advanced glycation end-products (AGEs). Excess production of AGEs was considered as an early marker of cardiac disease in type-2 DM with endothelial dysfunction. As hyperglycemia damages the vascular endothelium, which enhances the production of AGEs, increases the expression of AGE receptors (RAGE) and their ligands[49], increasing superoxide production decreasing NO synthesis that promotes vascular inflammation and dysfunction[50].

Yonekura *et al*.[51] has reported that circulating soluble isoforms of RAGE lack the intracellular and the intramembranous parts of the receptor regulating RAGE activity by competitive inhibition. Fujisawa *et al*.[52] has reported higher circulating sRAGE levels in type 2 diabetic patients that may be a counter-system against endothelial cell damage and could reflect enhanced RAGE expression in the diabetic vasculature. Additionally, a positive association between its level and the severity of the disease was reported by Nin *et al*.[53]. In support to our findings, Sherif *et al*.[54] found elevated sRAGE levels in diabetic patients with stable coronary artery diseases, suggesting its predictive value to the high risk of CVD development, as well as to evaluate the effects of potential therapeutic interventions. Moreover, the insignificant effect of folic acid post treatment in group (IIIb) on sRAGE could be explained by the possibility that sRAGE has different relationships in different states of DM, age, and level of AGEs/ RAGE expression[8]. Additionally, sherif *et al*.[54] reported that sRAGE might be influenced by the time of sampling and stage of the disease.

Notably, also it was found that compliance of the left ventricle decreases with myocardium accumulation of AGEs. The positive association found between sRAGE and cardiac function tests in men with and without diabetes, reflecting a direct role of the AGE-RAGE signaling on the left ventricular function[55]. This may explain why sRAGE is associated with the cardiac enzymes, ECG changes and ABP in this study.

The relation between sRAGE and incident HF risk are contradictory and argumentative, as Lazo *et al*.[56] showed a negative association between sRAGE and the incident of HF in middle-aged adults, this discrepancy with the results of the present work could be related to the difference in the species, stage of the disease and the intervention used.

Additionally, the significant reduction in MDA, SOD in folic acid protective group (IIIa) and the increased SOD in folic acid therapeutic group (IIIb) may reflect lower levels of ROS generation in those rats treated with folic acid in protective group (IIIa), or efforts generated by SOD to conflict OS in therapeutic group (IIIb) to which animals were chronically exposed that resulted in reduction in the activity of SOD with increase in MDA in untreated DCM rats (IIb) in the present work reflecting high oxidative pressure and the diminished antioxidant defense potential in long standing cases of untreated hyperglycemia and dyslipidemia, as SOD acts as a scavenger for toxic superoxide radical which is implicated in lipid peroxidation[35].

The previous results were confirmed by iNOS expression in cardiac tissue where there were significantly increased immune positive cells in DMC groups (IIa &IIb) compared to the control groups. these findings agreed with that of De Belder *et al*.[57] who stated that there was an excessive NO production in patients with cardiac dysfunction resulting from cytokine-induced expression of iNOS in myocardial and vascular tissues. The authors showed that expression of iNOS protein with a high prevalence of TNF-alpha protein expression in cardiac myocytes related with cardiomyopathy. Nagareddy *et al*.[58] reviewed that iNOS expression was significantly elevated in STZ diabetic rat hearts and it considered a predominant contributor to oxidative/nitrosative stress in diabetic myocardium as once induced, iNOS is known to generate large amounts of NO until the enzyme is degraded.

In folic acid protected group (IIIa) and therapeutic group (IIIb) there was a significant decrease in iNOS immune positive cells compared to those of the groups (IIa) and (IIb). Moreover, iNOS immune positive cells expression in group (IIIb) was significantly more than that in (IIIa). This result may be due to the mechanism of folic acid as antioxidant by suppressing of iNOS activity and expression, a decreased NO content, a reduction in the formation of lipid peroxides, and thereby a suppression of the OS injury to protect the myocardial tissue[59].

OS could be responsible for abnormal myocardial ultrastructural changes such as degeneration, interstitial

inflammation, and hemorrhage. In turn these abnormalities are responsible for further elevation of oxidative/ nitrogenous stress[4] that could be attributed to the presence of a significant hyperglycemia[60]. The histopathological finding of the present work agreed with these observations.

The coexisting of OS and inflammation in DCM groups in the present study could promote cardiac remodeling changes that characterize diabetic cardiomyopathy[61]. Interestingly, in Folic acid treated group, there was a significant decrease in CRP in comparison to DCM untreated groups, which was supported by Kim *et al*.[59] who reported that folic acid, has a role in decreasing inflammatory reactions through the anti-oxidative capacity of folate. In addition, high serum folate levels could improve vascular endothelial function and lower the risk of stroke and CVD[62]. However, there was an insignificant change in CRP between Group (IIIa) and Group (IIIb), indicating the prominent anti-inflammatory properties of folic acid supplementation.

Furthermore, it has been observed that IR and OS could activate cellular apoptotic- pathways including caspase-3 pathway that causes DNA fragmentation, cellular death, and necrosis[63] predisposing for myocardial cell injury and cardiomyopathy development[64].

This could explain the significant improvement in blood pressure in folic treated groups in comparison to untreated DCM groups in the present work, as folic acid was found to improve impaired vascular reactivity, increased peripheral vascular resistance and elevated blood pressure in conditions of abnormal glucose and lipid metabolism[65].

However, folic acid did not induce significant change in HR when compared to DCM untreated groups. That observation agreed with the result of Craig *et al*.[66] who noticed that, despite short-term folic acid supplementation protecting the heart from autonomic dysfunction, it has not altered HR.

The current study suggested that protective effects of folic acid treatment were more effective than the therapeutic ones in improving cardiac pathophysiological changes induced by type-2 DM. As there was a significant increase in PR interval, R amplitude, and a significant decrease in serum LDH, CK-MB, % area of collagen deposition and % area of caspase immune-positive cells in protective folic acid group (IIIa) when compared to therapeutic group (IIIb). However, ECG tracing in folic acid therapeutic group (IIIb) showed the presence of a pathological Q wave that is a marker of myocardial infarction, coronary atherosclerosis and increased cardiovascular risk and could be explained by endothelial insulin resistance and dysfunction[67].

This result may be attributed to better glycemic control, and the upper efficacy of protective administration of folic acid in scavenging of various free radicals. These observations were in accordance with Moens *et al*.[10] who demonstrated preserved myocardial function in folic acid pretreated rats subjected to regional coronary occlusion because of less generated superoxide. However, there are relatively few data on the effect of folate supplementation on chronic heart disease outcome. Furthermore, failure of therapeutic folic acid administration to decrease sRAGE could be explained by the difference in the onset of the therapy between both groups.

On the contrary, a study of Liem *et al*.[68] reported that a 2-yr intervention with low-dose folic acid (0.5 mg/day) had no beneficial effects. However, the dose of folic acid used was too low to produce any benefit.

CONCLUSION

In conclusion, the current study demonstrated that administration of folic acid in rats with experimentally induced diabetic cardiomyopathy has beneficial protective and therapeutic effects via exertion partial amelioration of HFD-STZ-induced diabetic cardiomyopathy by attenuating electrocardiogram, histological changes, cardiac tissue collagen deposition and apoptosis. These effects could be attributed to reducing glucose-metabolic disturbances, OS, and inflammation. In addition, protective therapy could target RAGE activation that influenced the pathogenesis and reflected on sRAGE levels. Indeed, therapeutic use of folic acid was not as effective as protective one. Therefore, the use of dietary supplements is of great importance, to prevent or reduce the DCM complications.

RECOMMENDATION

Data on the sequel of folic acid on the AGE–RAGE signaling are limited. Further studies are required to speculate the clinical importance of the use of folic acid in the context of sRAGE regulation, and additional mechanistic studies are needed as well as investigations of the sources and functions of sRAGE. Further clinical studies are also needed to establish the value of sRAGE as a prognostic marker in patients with DCM.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Lorenzo-Almorós A, Tuñón J, Orejas M, Cortés M, Egido J, Lorenzo Ó. Diagnostic approaches for diabetic cardiomyopathy. Cardiovascular diabetology. 2017 Dec;16(1):1-4. DOI: https://doi.org/10.1186/s12933-017-0506-x.

2. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. Circulation research. 2018 Feb 16;122(4):624-38. Doi: 10.1161/CIRCRESAHA.117.311586.

3. Athithan L, Gulsin GS, McCann GP, Levelt E. Diabetic cardiomyopathy: Pathophysiology, theories, and evidence to date. World journal of diabetes. 2019 Oct 15;10(10):490. Doi:10.4239/wjd. v10.i10.490

4. Murtaza G, Virk HU, Khalid M, Lavie CJ, Ventura H, Mukherjee D, Ramu V, Bhogal S, Kumar G, Shanmugasundaram M, Paul TK. Diabetic cardiomyopathy-A comprehensive updated review. Progress in cardiovascular diseases. 2019 Jul 1;62(4):315-26. DOI: 10.1016/j.pcad.2019.03.003.

5. D'Oria R, Schipani R, Leonardini A, Natalicchio A, Perrini S, Cignarelli A, Laviola L, Giorgino F. The role of oxidative stress in cardiac disease: from physiological response to injury factor. Oxidative medicine and cellular longevity. 2020 May 14;2020. DOI: https://doi.org/10.1155/2020/5732956.

6. Bayarsaikhan E, Bayarsaikhan D, Lee J, Son M, Oh S, Moon J, Park HJ, Roshini A, Kim SU, Song BJ, Jo SM. Microglial AGE-albumin is critical for neuronal death in Parkinson's disease: a possible implication for theranostics. International journal of nanomedicine. 2015;10(Spec Iss):281. DOI: 10.2147/IJN.S95077. eCollection 2015.

7. White DL, Hoogeveen RC, Chen L, Richardson P, Ravishankar M, Shah P, Tinker L, Rohan T, Whitsel EA, El-Serag HB, Jiao L. A prospective study of soluble receptor for advanced glycation end products and adipokines in association with pancreatic cancer in postmenopausal women. Cancer medicine. 2018 May;7(5):2180-91. DOI: 10.1002/cam4.1426.

8. Wannamethee SG, Welsh P, Papacosta O, Ellins EA, Halcox JP, Whincup PH, Sattar N. Circulating soluble receptor for advanced glycation end product: Cross-sectional associations with cardiac markers and subclinical vascular disease in older men with and without diabetes. Atherosclerosis. 2017 Sep 1; 264:36-43. DOI: 10.1016/j.atherosclerosis. 2017.07.008

9. 9. Mahmood L. The metabolic processes of folic acid and Vitamin B12 deficiency. Journal of Health Research and Reviews. 2014 Jan 1;1(1):5. DOI: 10.4103/2394-2010.143318.

10. Moens AL, Vrints CJ, Claeys MJ, Timmermans JP, Champion HC, Kass DA. Mechanisms, and potential therapeutic targets for folic acid in cardiovascular disease. American Journal of Physiology-Heart and Circulatory Physiology. 2008 May;294(5):H1971-7. DOI: https://doi.org/10.1152/ajpheart.91503.2007

11. Hou J, Yan G, Liu B, Zhu B, Qiao Y, Wang D, Li R, Luo E, Tang C. The protective effects of enalapril maleate and folic acid tablets against contrast-induced nephropathy in diabetic rats. BioMed research international. 2018 Feb 7;2018. DOI: 10.1155/2018/4609750. eCollection 2018.

12. Mutavdzin S, Gopcevic K, Stankovic S, Jakovljevic Uzelac J, Labudovic Borovic M, Djuric D. The effects of folic acid administration on cardiac oxidative stress and cardiovascular biomarkers in diabetic rats. Oxidative medicine and cellular longevity. 2019 Jun 11;2019. DOI: https://doi.org/10.1155/2019/1342549.

13. Nkemjika S, Ifebi E, Cowan LT, Fung IC, Twum F, Liu F, Zhang J. Association between serum folate and cardiovascular deaths among adults with hypertension. European journal of clinical nutrition. 2019 Nov 27:1-9. DOI: https://doi.org/10.1038/s41430-019-0533-7

14. Svegliati-Baroni G, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, De Minicis S, Nobili L, Salzano R, Omenetti A, Pacetti D. A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-α and n-3 polyunsaturated fatty acid treatment on liver injury. The American journal of pathology. 2006 Sep 1;169(3):846-60. DOI: 10.2353/ajpath.2006.050953

15. Ménard SL, Croteau E, Sarrhini O, Gélinas R, Brassard P, Ouellet R, Bentourkia MH, van Lier JE, Rosiers CD, Lecomte R, Carpentier AC. Abnormal in *vivo* myocardial energy substrate uptake in diet-induced type 2 diabetic cardiomyopathy in rats. American Journal of Physiology-Endocrinology and Metabolism. 2010 May;298(5): E1049-57. https://doi.org/10.1152/ajpendo.00560.2009

16. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacological research. 2005 Oct 1;52(4):313-20. DOI: 10.1016/j.phrs.2005.05.004.

17. Patel SS, Goyal RK. Cardioprotective effects of gallic acid in diabetes-induced myocardial dysfunction in rats. Pharmacognosy research. 2011 Oct;3(4):239. DOI: 10.4103/0974-8490.89743.

18. Moat SJ, Doshi SN, Lang D, McDowell IF, Lewis MJ, Goodfellow J. Treatment of coronary heart disease with folic acid: is there a future? American Journal of Physiology-Heart and Circulatory Physiology. 2004 Jul 1. DOI: 10.1152/ajpheart.00952.2003.

19. Zaafan MA, Zaki HF, El-Brairy AI, Kenawy SA. Protective effects of atorvastatin and quercetin on isoprenaline-induced myocardial infarction in rats. Bulletin of Faculty of Pharmacy, Cairo University. 2013 Jun 1;51(1):35-41. DOI:10.1016/j.bfopcu.2013.03.001

20. Pereira-Junior PP, Marocolo M, Rodrigues FP, Medei E, Nascimento JH. Noninvasive method for electrocardiogram recording in conscious rats: feasibility for heart rate variability analysis. Anais da Academia Brasileira de Ciências. 2010 Jun;82(2):431-7. DOI: https://doi.org/10.1590/S0001-37652010000200019

21. Finley PR, Tietz NW, editors. Clinical guide to laboratory tests. WB Saunders company; 1996.

22. Temple R, Clark PM, Hales CN. Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Diabetic medicine. 1992 Jul;9(6):503-12. DOI: 10.1111/j.1464-5491. 1992.tb01830. x.

23. Sun G, Bishop J, Khalili S, Vasdev S, Gill V, Pace D, Fitzpatrick D, Randell E, Xie YG, Zhang H. Serum visfatin concentrations are positively correlated with serum triacylglycerols and downregulated by overfeeding in healthy young men. The American journal of clinical nutrition. 2007 Feb 1;85(2):399-404. DOI: 10.1093/ajcn/85.2.399.

24. Moss DW, Henderson AR, Kachmar JF. Enzymes, In: Tietz NW, ed. Fundamentals of Clinical Chemistry. (3rd edn.). Philadelphia: WB Saunders; 1987. Pp: 346-421.

25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979 Jun 1;95(2):351-8. DOI: 10.1016/0003-2697(79)90738-3.

26. Kakkar P, Das B, Viswanathan P. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21: 130-132. PMID: 6490072.

27. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation. 1998 Aug 25;98(8):731-3. DOI: 10.1161/01.cir.98.8.731.

28. Falcone C, Emanuele E, D'Angelo A, Buzzi MP, Belvito C, Cuccia M, Geroldi D. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arteriosclerosis, thrombosis, and vascular biology. 2005 May 1;25(5):1032-7. DOI: 10.1161/01.ATV.0000160342.20342.00.

29. Bancroft J and Gamble M. Theory and Practice of histological techniques (6th edition). Churchil Livingstone, London 2008; 7: 165-175.

30. Laurentino SS, Correia S, Cavaco JE, Oliveira PF, Rato L, Sousa M, Barros A, Socorro S. Regucalcin is broadly expressed in male reproductive tissues and is a new androgen-target gene in mammalian testis. Reproduction. 2011 Sep 1;142(3):447. DOI: 10.1530/rep-11-0085

31. Heger J, Gödecke A, Flögel U, Merx MW, Molojavyi A, Kühn-Velten WN, Schrader J. Cardiac-specific overexpression of inducible nitric oxide synthase does not result in severe cardiac dysfunction. Circulation research. 2002 Jan 11;90(1):93-9. https://doi.org/10.1161/hh0102.102757

32. Varghese F, Bukhari AB, Malhotra R, De A. IHC Profiler: an open-source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. PloS one. 2014 May 6;9(5): e96801. DOI: 10.1371/journal.pone.0096801.

33. Guo XX, Wang Y, Wang K, Ji BP, Zhou F. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. Journal of Zhejiang University-Science B. 2018 Jul;19(7):559-69. DOI: 10.1631/jzus. B1700254.

34. Sabourin J, Robin E, Raddatz E. A key role of TRPC channels in the regulation of electromechanical activity of the developing heart. Cardiovascular research. 2011 Nov 1;92(2):226-36. DOI: https://doi.org/10.1093/cvr/cvr167

35. Vinik AI, Casellini C, Parson HK, Colberg SR, Nevoret ML. Cardiac autonomic neuropathy in diabetes: a predictor of cardiometabolic events. Frontiers in neuroscience. 2018 Aug 27;12: 591. DOI: doi: 10.3389/fnins.2018.00591.

36. Upaganlawar A, Balaraman R. Protective Effects of Lagenaria siceraria (Molina) Fruit Juice in Isoproterenol Induced Myocardial Infarction. International Journal of Pharmacology. 2010 Sep 1;6(5):645-51. DOI: 10.3923/ijp.2010.645.651

37. Elsawy M, Emara E. The impact of ghrelin on oxidative stress and inflammatory markers on the liver of diabetic rats. Tanta Medical Journal. 2016 Oct 1;44(4):163. DOI: 10.4103/1110-1415.201723.

38. Saleem MT, Chetty MC, Kavimani S. Putative antioxidant property of sesame oil in an oxidative stress model of myocardial injury. Journal of cardiovascular disease research. 2013 Sep 1;4(3):177-81. DOI: 10.1016/j.jcdr.2013.07.001

39. Thent ZC, Lin TS, Das S, Zakaria Z. histological changes in the heart and the proximal aorta in streptozotocin-induced diabetic rats following piper sarmentsoum administration. African Journal of Traditional, Complementary and Alternative Medicines. 2012 Feb 3;9(3):396-404. PMCID: PMC3746658

40. Wu W, Liu X, Han L. Apoptosis of cardiomyocytes in diabetic cardiomyopathy involves overexpression of glycogen synthase kinase-3β. Bioscience reports. 2019 Jan 31;39(1). doi: 10.1042/BSR20171307. PMCID: PMC6328876

41. Yu W, Wu J, Cai F, Xiang J, Zha W, Fan D, Guo S, Ming Z, Liu C. Curcumin alleviates diabetic cardiomyopathy in experimental diabetic rats. PloS one. 2012 Dec 14;7(12): e52013. PMCID: PMC3522633. DOI: 10.1371/journal.pone.0052013

42. Abdel-Hamid AA, Firgany AE. Atorvastatin alleviates experimental diabetic cardiomyopathy by suppressing apoptosis and oxidative stress. Journal of molecular histology. 2015 Oct;46(4):337-45. DOI: 10.1007/s10735-015-9625-4

43. Soldani C, Scovassi AI. Poly (ADP-ribose) polymerase-1 cleavage during apoptosis: an update. Apoptosis. 2002 Aug;7(4):321-8. DOI: 10.1023/a:1016119328968

44. Stanhewicz AE, Kenney WL. Role of folic acid in nitric oxide bioavailability and vascular endothelial function. Nutrition reviews. 2017 Jan 1;75(1):61-70. DOI: 10.1093/nutrit/nuw053

45. Li W, Tang R, Ouyang S, Ma F, Liu Z, Wu J. Folic acid prevents cardiac dysfunction and reduces myocardial fibrosis in a mouse model of high-fat diet-induced obesity. Nutrition & metabolism. 2017 Dec;14(1):1-8. DOI: 10.1186/s12986-017-0224-0

46. Shafikhani SH, Morales C, Engel J. The Pseudomonas aeruginosa type III secreted toxin ExoT is necessary and sufficient to induce apoptosis in epithelial cells. Cellular microbiology. 2008 Apr;10(4):994-1007. https://doi.org/10.1111/j.1462-5822.2007.01102.x

47. Liu YS, Huang ZW, Wang L, Liu XX, Wang YM, Zhang Y, Zhang M. Sitagliptin alleviated myocardial remodeling of the left ventricle and improved cardiac diastolic dysfunction in diabetic rats. Journal of Pharmacological Sciences. 2015 Mar 1;127(3):260-74. DOI: 10.1016/j.jphs.2014.12.007

48. Yamagishi SI, Nakamura N, Suematsu M, Kaseda K, Matsui T. Advanced glycation end products: a molecular target for vascular complications in diabetes. Molecular Medicine. 2015 Jan;21(1): S32-40. DOI: 10.2119/molmed.2015.00067

49. Kaur R, Kaur M, Singh J. Endothelial dysfunction, and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. Cardiovascular diabetology. 2018 Dec;17(1):1-7. DOI: 10.1186/s12933-018-0763-3

50. Selvin E, Halushka MK, Rawlings AM, Hoogeveen RC, Ballantyne CM, Coresh J, Astor BC. sRAGE and risk of diabetes, cardiovascular disease, and death. Diabetes. 2013 Jun 1;62(6):2116-21. DOI: 10.2337/db12-1528

51. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, ABEDIN M, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochemical Journal. 2003 Mar 15;370(3):1097-109. DOI: 10.1042/BJ20021371

52. Fujisawa K, Katakami N, Kaneto H, Naka T, Takahara M, Sakamoto F, Irie Y, Miyashita K, Kubo F, Yasuda T, Matsuoka TA. Circulating soluble RAGE as a predictive biomarker of cardiovascular event risk in patients with type 2 diabetes. Atherosclerosis. 2013 Apr 1;227(2):425-8. DOI: 10.1016/j.atherosclerosis.2013.01.016.

53. Nin JW, Jorsal A, Ferreira I, Schalkwijk CG, Prins MH, Parving HH, Tarnow L, Rossing P, Stehouwer CD. Higher plasma soluble receptor for advanced glycation end products (sRAGE) levels are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study. Diabetes. 2010 Aug 1;59(8):2027-32. DOI: 10.2337/db09-1509.

54. Sherif EM, Abdelmaksoud AA, Issa HM, Mohamed SA. Soluble receptor for advanced glycation end products (sRAGE) and carotid intima-media thickness (CIMT) in type 1 diabetes Mellitus: Possible association with diabetic vascular complications. Egyptian Journal of Medical Human Genetics. 2014 Oct 23;15(4):361-7. https://doi.org/10.1016/j.ejmhg.2014.06.003

55. Hartog JW, Voors AA, Bakker SJ, Smit AJ, van Veldhuisen DJ. Advanced glycation end-products (AGEs) and heart failure: pathophysiology and clinical implications. European journal of heart failure. 2007 Dec;9(12):1146-55. DOI: 10.1016/j.ejheart.2007.09.009.

56. Lazo M, Halushka MK, Shen L, Maruthur N, Rebholz CM, Rawlings AM, Hoogeveen RC, Brinkley TE, Ballantyne CM, Astor BC, Selvin E. Soluble receptor for advanced glycation end products and the risk for incident heart failure: the atherosclerosis risk in communities' study. American heart journal. 2015 Nov 1;170(5):961-7. DOI: 10.1016/j.ahj.2015.08.008

57. De Belder AJ, Radomski MW, Martin JF, Moncada S. Nitric oxide, and the pathogenesis of heart muscle disease. European journal of clinical investigation. 1995 Jan;25(1):1-8. https://doi.org/10.1111/j.1365-2362.1995.tb01517.x

58. Nagareddy PR, Xia Z, McNeill JH, MacLeod KM. Increased expression of iNOS is associated with endothelial dysfunction and impaired pressor responsiveness in streptozotocin-induced diabetes. American Journal of Physiology-Heart and Circulatory Physiology. 2005 Nov;289(5):H2144-52. https://doi.org/10.1152/ajpheart.00591.2005

59. Kim T, Oak CH, Jung MH, Jang TW, Kim J. High Serum Folate Concentration Is Associated with Better Lung Function in Male Chronic Obstructive Pulmonary Disease Patients Who Are Current Smokers: Analysis of Nationwide Population-Based Survey. Nutrients. 2020 Aug;12(8):2219. DOI: 10.3390/nu12082219.

60. Ansley DM, Wang B. Oxidative stress, and myocardial injury in the diabetic heart. The Journal of pathology. 2013 Jan;229(2):232-41. DOI: 10.1002/path.4113

61. Frati G, Schirone L, Chimenti I, Yee D, Biondi-Zoccai G, Volpe M, Sciarretta S. An overview of the inflammatory signalling mechanisms in the myocardium underlying the development of diabetic cardiomyopathy. Cardiovascular Research. 2017 Mar 15;113(4):378-88. DOI: 10.1093/cvr/cvx011.

62. Naderi N, House JD. Recent developments in folate nutrition. Advances in food and nutrition research. 2018 Jan 1;83: 195-213. DOI: 10.1016/bs.afnr.2017.12.006

63. Mohammed NA, El-Malkey NF, Ibrahim AA, Abdullah DM. Vitamin D3 supplementation ameliorates ovariectomy-induced cardiac apoptotic and structural changes in adult albino rats. Canadian journal of physiology and pharmacology. 2019;97(7):647-54. DOI: 10.1139/cjpp-2018-0674.

64. Wang X, Guo Z, Ding Z, Mehta JL. Inflammation, autophagy, and apoptosis after myocardial infarction. Journal of the American Heart Association. 2018 Apr 21;7(9): e008024. DOI: 10.1161/JAHA.117.008024.

65. Petrie JR, Guzik TJ, Touyz RM. Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. Canadian Journal of Cardiology. 2018 May 1;34(5):575-84. DOI: 10.1016/j.cjca.2017.12.005.

66. Craig J, Akam EC, Bailey SJ, Cox NJ, Ferguson RA, O'Donnell E. Effects of Short-term Folic Acid Supplementation on Brachial Artery Endothelial Function in Habitually Trained and Untrained Postmenopausal Women. The FASEB Journal. 2020 Apr 1;34(S1):1-. DOI: https://doi.org/10.1096/fasebj.2020.34.s1.08662

67. Bhatt AB, Mulvey CK, Qasim AN, Nair JV, Rickels MR, Prenner SB, Iqbal N, Reilly MP. Selective association of electrocardiographic abnormalities with insulin sensitivity and beta-cell function in type 2 diabetes mellitus: a cross-sectional analysis. Diabetes/metabolism research and reviews. 2016 Oct;32(7):736-44. DOI:10.1002/dmrr.2794

68. Liem A, Reynierse-Buitenwerf GH, Zwinderman AH, Jukema JW, van Veldhuisen DJ. Secondary prevention with folic acid: effects on clinical outcomes. Journal of the American College of Cardiology. 2003 Jun 18;41(12):2105-13. DOI: 10.1016/s0735-1097(03)00485-6.

الملخص العربى

التأثيرات الوقائية والعلاجية لحمض الفوليک على التغيرات القلبية التركيبية والكهربائية فى نموذج الجرذ لاعتلال عضلة القلب السكرى

نانيس فؤاد المالكي[١]، مريم نصر ميخائيل[١]، ماهر نجيب ابراهيم[١]، رانيا سعيد معوض[٢]، مها محمد أحمد عبد الرحمن[٢] وابتسام محمد إبراهيم[١]

[١]قسم الفسيولوجيا الطبية، [٢]قسم التشريح البشري وعلم الأجنة، كلية الطب ، جامعة الزقازيق ، مصر

خلفية البحث: اعتلال عضلة القلب السكري (DCM) هو اضطراب في القلب يمكن أن يتطور إلى فشل القلب. وهناك بيانات متضاربة حول دور حمض الفوليك في علاج اعتلال عضلة القلب السكري.

الهدف من الدراسة: صممت هذه الدراسة لمقارنة التأثيرات الوقائية والعلاجية لحمض الفوليك على مخطط كهربية القلب والتغيرات التركيبية لنموذج اعتلال عضلة القلب السكري في الجرذان.

طرق ومواد البحث: تم تقسيم ستين جرذا على النحو التالي: المجموعات الضابطة (Ia و Ib) ، ومجموعات اعتلال عضلة القلب السكري (IIa و IIb): تلقت الجرذان نظام غذائي عالي الدهن لمدة ٤ أسابيع ثم تم حقنها بالستربتوزوتوسين (٢٥ مجم / كجم / في الغشاء البروتوني) ، و المجموعات المعالجة بحمض الفوليك (IIIa و IIIb): تلقت الجرذان ٥ مجم / كجم يوميا عن طريق الفم لمدة ٨ أسابيع من وقت التأكد من الإصابة بمرض السكري أو نهاية الأسبوع السابع على التوالى. تم تقييم تخطيط القلب ، وضغط الدم ، ونزعة هيدروجين اللاكتات(LDH) ، والكرياتين كيناز- MB ، والمستقبلات القابلة للذوبان لنواتج الغلوزة النهائية المتقدمة (sRAGE) ، والإجهاد التأكسدي (OS) ودلالات الالتهاب. وتم دراسة تركيب نسيج البطين الأيسر باستخدام صبغة الهيماتوكسيلين والأيوسين وصبغة ماسون ثلاثى الكروم ، والتحليل الكيميائي المناعي لتعبير iNOS و٣Caspase.

النتائج: حدوث تحسن ذي دلالة احصائية في سعةالموجة R وانحراف المقطع ST وانخفاض في مستوي المستقبلات القابلة للذوبان لنواتج الغلوزة النهائية المتقدمة (sRAGE) في مجموعة حمض الفوليك الوقائية وليس العلاجية عند مقارنتها بمجموعة اعتلال عضلة القلب السكري غير المعالجة. علاوة على ذلك قد تحسنت إنزيمات القلب والتغيرات التركيبية وترسب ألياف الكولاجين ودلالات الإجهاد التأكسدي والالتهاب ، وموت الخلايا المبرمج تحسنا ذا دلالة احصائية في المجموعتين المعالجتين بحمض الفوليك بالمقارنة مع مجموعات اعتلال عضلة القلب السكري غير المعالجة.

الخلاصة: كان الدور الوقائي لحمض الفوليك أكثر فاعلية من الدور العلاجي على إصابة القلب في نموذج اعتلال عضلة القلب السكري في الجرذان. و ذلك يمكن أن يعزى إلى تحكم أفضل في نسبة السكر في الدم والإجهاد التأكسدي وتحسين إشارات AGE / RAGE وتحسين التغيرات التركيبية لنسيج عضلة القلب.