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ORIGINAL ARTICLE

Effect of Induced Hypoglycemia on Postnatal Heart Structure in Male Albino Rats

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ABSTRACT

Background: Hypoglycemia is a life-threatening stress, often seen in neonatal age because of inborn hyperinsulinism, inherited disorder in sugar metabolism and vigorous insulin treatment for juvenile-onset Diabetes Mellitus. Recurrent hypoglycemia typically disturbs cardiovascular function. Therefore this study aimed to explore the consequences of hypoglycemia on the postnatal heart structure in male albino rats.

Methods: Thirty six male albino rat pups at postnatal week 2(PNW2) were randomly allocated into two groups; control (GroupI) and treated (GroupII). GroupI was further split equally into control negative (gained no medicines) and control positive (injected Sc with 0.3 ml saline 3 times/week). Group II were injected Sc with human regular insulin 3 IU/kg body weight, 3 times/ week. Each of the previous groups were extra split according to age of animal sacrificing (at either PNW3 or PNW11) into 2 subgroups. Heart specimens were managed for light microscope, immuno-histochemical and morphometric studies. Blood samples were got for cardiac enzyme estimation.

Results: Induced hypoglycemia resulted in histological changes in the normal heart structure manifested by apparently disarrayed hypertrophied cardiac muscle fibers, vascular congestion and increased connective tissue cells. Also, the cardiomyocyte cytoplasm exhibited cytoplasmic vacuolization and rarefaction. Moreover, nuclear pyknosis and pleomorphism were obvious. Comparable to control groups, increased collagen fiber deposition, declined glycogen content, weakened desmin and increased P53 immune expressing, elevated serum cardiac and decreased antioxidant enzymatic levels were manifested. These findings were evident at PNW11 hypoglycemic group.

Conclusions: Intermittent hypoglycemia had detrimental consequences on the postnatal heart structure via oxidative stress.

Key words: Heart; Insulin; Hypoglycemia.

INTRODUCTION

The chief cause of death in people with diabetes remains cardiovascular disease (CVD) **[1]**. The hazard of CVD in adults with diabetes is at least two to four times the hazard in adults without diabetes **[2].** Due to the risk of iatrogenic hypoglycemia, the treatment and managing of diabetes remains suboptimal. There are about two episodes of symptomatic hyp oglycemia every week and at least 1 episode of se vere, transitory disabling hypoglycemia every yea r in people with type1 diabetes **[3]**. It has been known that insulin-mediated hypoglycemic events account for an average of 100,000 emergency department visits per year **[4]**. Besides causing coma, seizures, and brain injure, severe hypoglycemia can be fatal [**5**], accounting for up to 10% of deaths amongst young people with Type 1 diabetes [**6**]. Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia and turbulence in carbohydrate, fat and protein metabolism. It is associated with a total or relative deficiency in insulin secretion (DM1) or with insulin resistance (DM2) **[7]**. Severe hypoglycemia is a common trouble in insulin-treated type 2 diabetes and that the prevalence increases with duration of insulin therapy. However, evidence for CV morbidity associated with hypoglycemia has been mostly theoretical **[8]**. In (DM2), there were great manifestations concerning correlation between severe hypoglycemia and CVD such as myocardial infarction, heart failure and stroke **[9]**.When a hypoglycemic event occurs, sympathetic overactivity might occur causing disruption of atherosclerotic plaques with subsequent cardiac arrhythmia, vascular

dysfunction, and inflammation **[10]**. Neonatal hypoglycemia is the most common metabolic disorder that has a catastrophic impact on neonates and infants in early life. It was suggested that, severe hypoglycemia might affect cardiovascular function. Inborn hyperinsulinism, inherited fault in sugar metabolism and vigorous insulin treatment for (DM-1) constitute the main reasons of hypoglycemia encountered early in life **[11].** Neonatal hypoglycemia may be symptomatic with incidents of sweating, paleness, hypothermia, shakes, exhaustion, stupor and/or spasms. If not recognized and treated carefully, it may lead to toxic neurologic and cardiovascular outcomes **[12].**Regarding the hypoglycemic effect on the development of varying body organs, scarce studies have been met in the literature chiefly for the brain **[13].**Therefore, it is timely to assess the impact of hypoglycemia on the CV system, how this main metabolic stress could precipitate major vascular events for instance myocardial infarction and stroke, and its potential role in these new clinical studies. Thus, the main purpose of the present study was to observe the impact of hypoglycemia on postnatal heart structure at different developmental periods.

METHODS

Animals *:*In rats, the pregnancy period lasts for 21–23 days and is followed by a suckling period of about 21 days. Therefore 10 adult male rats with 20 adult female rats of 160-200 gm were purchased from the animal house, Faculty of Medicine, Zagazig University, Egypt. All the rats underwent an adaptation period for 1 week before the beginning of the study and were housed in separate stainless steel cages under air-conditioned room at 26° c with satisfactory ventilation, a 12-hrs light/dark cycle and were allowed the standard diet and water ad libitum all over the study period. Every one male rat was put with two female rats in separate cage overnight till pregnancy occurred. Presence of sperms in vaginal smear indicates the first day of pregnancy. An overall of 36 healthy male albino rat pups at postnatal day 14 weighting 70-100 g were taken randomly for the study. Ethical approval was obtained from the animal care committee of the National Research Center **(IACUC)** with Approval number (**ZU-IACUC/3/F/127/2020)**, Zagazig University, Egypt. The pups from week 1 till week 3 were kept with their mothers for suckling till the age of weaning (21st day postnatal), after which the pups were housed in separate cages under the same environmental conditions as their mothers and were allowed the standard diet and water ad libitum throughout the study period.

Study design *:*The rats were divided into 2 groups; control **(I)** and treated **(II)** groups. The **first group (I)** consisted of 24 pups that were splitted into 2 equal subgroups, each contained 12 pups: Control negative group in which no treatment was obtained and was further subdivided in accordance with age of animal sacrifice into 6 rats sacrificed at 3 weeks postnatal **(Ia) (representing the neonatal period**) and the other 6 at 11weeks postnatal **(Ib) (representing the young adult period)** as it was found that offspring rats of two weeks age were (equivalent to that of full term human newborns) **[14]**. Control positive group which was injected Sc with 0.3 ml normal saline three times a week and was also further subdivided in accordance with age of animal sacrifice into 6 pups sacrificed at 3 weeks postnatal**(Ic)** and the other 6 at 11weeks postnatal**(Id)**. The **second group** was the hypoglycemic group **(II)** contained 12 pups which were injected with 3 IU/kg of human regular insulin (Novo Nordisk Inc., A/S Denmark) dissolved in 0.3ml normal saline Sc three times per week **[15]**. Half of animals (6) were sacrificed at 3 weeks postnatal **(IIa)** and the other half at 11 weeks postnatal **(IIb)** as illustrated in **table (1)**. The body weight was measured before each injection to readjust the dose of insulin. The rats in the control groups (Ia) and (Ic) and the hypoglycemic group (IIa) sacrificed at three weeks postnatal were injected for one week only as they were obtained at the age of two weeks postnatal. While the rats in the control groups (Ib) and (Id) and the hypoglycemic group (IIb) sacrificed at 11 weeks postnatal were injected for 9 weeks only as they were obtained at the age of two weeks postnatal. The fasting blood-glucose level was estimated from the tail vein using Accu-Check Advantage glucometer two hours following Insulin injection. Rats with 40-55 mg/dl blood glucose level were considered hypoglycemic. This level is commonly used to match hypoglycemia in human newborns **[16].** All the rats were anesthetized and sacrificed using intraperitoneal injection of thiopental 50 mg/kg **(IACUC, 2013) [17].** The thoracic cavity was opened and the hearts (approximately 2 cm long) were dissected. The adherent adipose tissue and connective tissue were removed. The left ventricle was dissected longitudinally for the histological examination.

Histological Study *:*For the light microscopic study, tissues were processed routinely in paraffin wax embedding. The paraffin sections were dewaxed, rehydrated then sections of 6 um thick were sliced and stained with H&E stain to demonstrate the histological details, Periodic acid Schiff's (PAS) stain to show glycogen content and Masson's Trichrome (MT) staining to observe collagen fibers **[18]**. The sections were examined under a LEICA research microscope (LEICA Dm750, Switzerland) with a digital camera

attached (LEICA ICC50). Digital photomicrographs of stained sections were taken.

Immuno-histochemical staining: Paraffin sections of cardiac muscle and monoclonal antibody (RD301) against desmin were used. Clone, RD301 is a mouse monoclonal IgG2b antibody and reacts entirely with desmin and is expressed in smooth, cardiac, and striated muscle cells, and was obtained from (Thermo Fisher Scientific Industries). Avidin biotinylated horseradish peroxidase complex (ABC) technique is applied as detection reagent. Colour reaction was developed by using diamino–benzidine (DAB) and gave a brown colour to desmin. Haematoxylin was used for counterstaining **[19].** Furthermore, the Paraffin sections of cardiac tissue were stained with anti-p53 Immunoperoxidase stain for the evaluation of anti p53expression of the nuclei, which is considered as positive marker of apoptotic myofibril **[20]**.

Biochemical study and antioxidant activity

Animals of all groups were sacrificed at the end of the experiment. Blood samples were collected from the retro-orbital plexus; serum obtained by centrifugation at 3000 rpm for 10 min for the estimation of cardiac enzymes as creatine phosphokinase (CPK), Creatine kinase (CK-MB) and Lactate dehydrogenase (LDH). In addition, part of heart tissue was homogenized in 0.1M phosphate buffer and centrifuged at 4000 rpm for 15 min in a cooling centrifuge and the supernatant was pipetted into plastic tubes for determination of antioxidants such as catalase (CAT) enzyme, Glutathione (GSH) , Oxidative stress biomarkers, lipid peroxidation was evaluated by measuring malondialdhyde (MDA) level**[21]**.

Image analysis and morphometry

Image analysis and morphometry was performed by ImageJ software (Wayne Rasband, National Institute of Mental Health, Bethesda, Maryland, USA). All the parameters were done at Human Anatomy and Embryology Department, Faculty of Medicine, Zagazig University. The mean area percentages of collagen and glycogen were evaluated in sections stained by Masson's Trichrome and PAS stains at x400 magnification respectively. As well as, in sections marked by Desmin, the mean area percentages of intercalated discs were assessed at x400 magnification. In addition, apoptosis was quantified by measuring the mean area percentage of P53 positive cells at x400 magnification in each field. Five fields per slide and five slides per animal were evaluated for each parameter.

STATISTICAL ANALYSIS

The collected data were computerized and statistically analyzed using Graph Pad Prism 5.01. Quantitative data were expressed as mean ± SD (Standard deviation), differences between mean values of experimental groups were tested with t test and the results were considered statistically significant when the P value <0.05. Different stages of significance were considered. High significance (***) when P value < 0.001, Moderate significant (**) at 0.01 >P value >0.001 and low significance (*) when 0.05 >P value >0.01

RESULTS

Histological Study

Light microscopic examination

Control (I) group at 3 weeks

Histological examination of all sections from rats of control subgroups **(Ia) and (Ic)** revealed no documented histological differences between them. Thus, the results of subgroup **(Ia)** were chosen to describe both subgroups. Light microscopic examination of the H&E stained sections of left ventricular tissue of both control subgroups **(Ia) and (Ic)** sacrificed at 3 weeks postnatal showed regularly arranged bundles of cardiac muscle fibers with single oval centrally located nuclei. In addition, blood capillaries and fibroblasts were seen in the connective tissue between cardiac muscle fibers **(Fig. 1A).** Also, sections of left ventricular tissue stained with Masson's trichrome revealed normal distribution of green colored collagen fibers in the endomysium between the cardiac muscle fibers and around the blood vessels **(Fig.3A)**. In addition, the glycogen distribution in the cardiac muscle of both control subgroups was stained strong pink colour with PAS stain **(Fig.3C)**. Furthermore, Immunohistochemical staining for desmin showed apparent brown colour in the intercalated discs of cardiac muscle fibers in the control subgroups **(Fig.3E)**. Also, Heart sections of the control subgroups stained with P53 immunostaining showing minimal anti- P53 expression of the nuclei within the cardiac muscle fibers **(Fig.3G)**.

Hypoglycemic (II) group at 3 weeks

Light microscopic examination of the H&E stained sections of left ventricular tissue of hypoglycemic group sacrificed at 3 weeks postnatal showed mild degenerating changes when compared with control subgroups. Mildly disarrayed and separated bundles of cardiac muscle fibers were well demonstrated. Some muscle fibers showed deep acidophilic cytoplasm with dark stained nuclei. Other fibers appeared with pale acidophilic cytoplasm and peri nuclear empty spaces **(vacuolated cytoplasm)**. Some bizarre shaped nuclei and dilated CT spaces were distinguished. Mildly dilated and congested blood capillaries, increased connective tissue (CT) cells between cardiomyocytes and around some blood capillaries and few extravasated RBCs were noticed **(Fig. 1 [B-D])**. Also, sections of left ventricular tissue

stained with Masson trichrome revealed mildly increased deposition of collagen fibers in the endomysium of cardiomyofibers and around the blood vessels when compared with control subgroups **(Figs.3B)**. In addition, the glycogen distribution in the cardiac muscle showed mild reduction in the distribution of glycogen materials in the sections stained with PAS **(Fig.3D)**. Furthermore, a mild reduction in desmin immunoreactivity in the intercalated discs between cardiomyocytes was obviously seen as mild brown coloration **(Fig.3F)**. Also, Heart sections of the hypoglycemic group stained with P53 immunostaining showing mildly increased anti-P53 expression of the nuclei within the cardiac muscle fibers **(Fig.3H)**.

Control (I) group at 11 weeks :Histological examination of all sections from rats of control subgroups **(Ib) and (Id)** revealed no documented histological differences between them. Thus, the results of subgroup **(Ib)** were chosen to describe both subgroups. Light microscopic examination of the H&E stained sections of left ventricular tissue of both control subgroups **(Ib) and (Id)** sacrificed at 11 weeks postnatal showed normal architecture in the form of regularly arranged bundles of cardiac muscle fibers with apparently single oval smaller size centrally located nuclei. In addition, blood capillaries and fibroblasts were seen in the connective tissue between cardiac muscle fibers **(Fig.2A).** Also, sections of cardiac tissue stained with Masson's trichrome revealed normal distribution of green colored collagen fibers in the endomysium between the cardiac muscle fibers and around the blood vessels **(Fig.4A)**. Additionally, PAS stained sections showed deep pink coloured glycogen normally distributed in the cardiac muscle fibers of both control subgroups **(Fig.4C)**. Furthermore, Immunohistochemical staining for desmin showed evident brown colour in the intercalated discs of cardiac muscle fibers in the control subgroups **(Fig.4E)**. Also, Heart sections of the control subgroups stained with P53 immunostaining showing minimal anti- P53 expression of the nuclei within the cardiac muscle fibers **(Fig.4G)**.

Hypoglycemic (II) group at 11 weeks

Light microscopic examination of the H&E stained sections of left ventricular tissue of hypoglycemic group sacrificed at 11 weeks postnatal showed great degenerative changes when compared with the corresponding group sacrificed at 3 weeks postnatal. Disrupted normal architecture in the form of disorientation of cardiac muscle bundles, apparently increased size of cardiac muscle fibers (hypertrophy) in comparison with the group sacrificed at 3 weeks postnatal and deeply stained (pyknotic) nuclei were observed. In addition, many cardiac muscle fibers with (pale acidophilic cytoplasm) donating decreased myofilaments **(rarified cytoplasm)**, others with markedly vacuolated cytoplasm, numerous discrete CT cells and severely congested blood vessels were seen. Bizarre shaped nuclei with perinuclear empty spaces were noticed **(Fig.2 [B-D])**. Also, sections of cardiac tissue stained with Masson's trichrome revealed markedly increased deposition of collagen fibers in the endomysium of cardiomyofibers and around the blood vessels in comparison with the control groups **(Fig.4B).** In addition, the glycogen distribution in the cardiac muscle showed marked decline in the distribution of glycogen materials in the sections stained with PAS in comparison with the control groups **(Fig.4D)**. Furthermore, Immunohistochemical staining for desmin showed a weak positive immunoreactivity of desmin of cardiomyocytes and expressed faint brown colour in intercalated discs in comparison with the control groups **(Fig.4F)**. Also, Heart sections of the hypoglycemic group stained with P53 immunostaining showing marked increase of anti- P53 expression of the nuclei within the cardiac muscle fibers in comparison with the control groups **(Fig.4H)**.

Morphometric Study and Statistical Analysis

Considering glycogen content and collagen area percentage, the results of the present study showed a highly significant difference ($P< 0.001$) in the area percentage of glycogen content stained with PAS and collagen fibers stained with Masson trichrome in both hypoglycemic groups sacrificed at 3 and 11 weeks postnatal relative to their control groups. Concerning anti- P53 expression of the nuclei within the myocardial fibers, the present study showed a highly significant increase ($P< 0.001$) in hypoglycemic group sacrificed at 11 weeks postnatal when compared with its control group. While there is no significant difference ($P> 0.05$) in anti- P53 expression of the nuclei in hypoglycemic group sacrificed at 3 weeks postnatal when compared with its control group. Additionally, desmin immunostaining of the cardiac muscle fibers revealed highly significant decrease ($P< 0.001$) in hypoglycemic group sacrificed at 11 weeks postnatal when compared with its control group, and moderately significant decrease($P< 0.01$) in hypoglycemic group sacrificed at 3 weeks postnatal when compared with its control group as shown in **table (2)** and **figure (5)**.

Biochemical study and antioxidant activity

The results of the present study showed a highly significant increase in serum levels of cardiac enzymes **[CPK, (CK-MB) and LDH]** ($p < 0.001$) in the hypoglycemic groups sacrificed at 3 weeks and 11 weeks postnatal respectively when

compared with control groups as shown in **table (3)** and **figure (6)**. As regard antioxidant activity, **Catalase (CAT)** antioxidant enzyme activity and **Glutathione (GSH)** levels in the heart tissue were highly significantly decreased ($p < 0.001$) in both hypoglycemic groups when compared with its values in control ones, while the lipid peroxidation evaluated by **Malondialdhyde (MDA)** level was highly significantly increased ($p < 0.001$) in the heart of both hypoglycemic groups when compared with the normal control groups. This fact confirmed the established oxidative stress in the cardiomyocytes associated with hypoglycemia as shown in **table (3)** and **figure (6)**.

Table 1: Illustration of the different groups used in the study design

36 male albino rat pups were used			
Group1 (Negative Control Group): consisted of 12 male albino rat pups and further subdivided according to age of animal sacrifice into 6 rats sacrificed at 3 weeks postnatal (Ia) and the other 6 at 11weeks postnatal (Ib)	**Group 2 (Positive Control) Group**: consisted of 12 male albino rat pups and were injected Sc with 0.3 ml normal saline three times per week and further subdivided according to age of animal sacrifice into 6 pups sacrificed at 3 weeks postnatal **(Ic)** and the other 6 at 11weeks postnatal **(Id).**	**Group 3 (Hypoglycemic group):** consisted of 6 male albino rat pups and were injected with 3 IU/kg of human regular insulin Sc three times per week then were sacrificed at 3 weeks postnatal **(IIa) [14].**	**Group4 (Hypoglycemic group):** consisted of 6 male albino rat pups and were injected with 3 IU/kg of human regular insulin Sc three times per week then were sacrificed at 11 weeks postnatal **(IIb) [14].**

Table 2: Morphometrical analysis of different parameters in cardiac muscle of different studied age groups (3 and 11 weeks postnatal)

	3 weeks			**11 weeks**		
	Control	**Hypoglycemia**	**P value**	**Control**	**Hypoglycemia**	**P value**
Masson	3.52±0.398	19.43±3.361	< 0.0001	4.69±0.658	29.52±3.491	< 0.0001
PAS	78.25±1.237	62.87±2.068	< 0.0001	81.45±2.347	53.33±3.427	< 0.0001
Desmin	13.48±1.575	11.02±0.769	0.0014	13.45±1.686	3.03±0.461	< 0.0001
P53	0.36±0.062	0.53±0.295	0.1466	1.49±0.307	2.85±0.463	< 0.0001

Analysis was done by t test. Data are presented as mean ± SD. The results were considered statistically significant when the P value <0.05.

Table 3: Biochemical and antioxidant activity analysis in different studied age groups (3 and 11 weeks postnatal):

	3 weeks			**11 weeks**		
	Control	**Hypoglycemia**	**P value**	**Control**	**Hypoglycemia**	**P value**
CPK (U/L)	60.6±4.4	86.2±4.9	< 0.0001	59.4±2.6	104.9±4.3	< 0.0001
CK-MB (U/L)	27.2±5.1	51.1±5.1	< 0.0001	29.6±3.4	69.8±3.1	< 0.0001
LDH (U/L)	217.8±4.7	244.0±7.5	< 0.0001	218.3±3.9	270.0±5.9	< 0.0001
CAT (U/ g. tissue)	53.5±4.7	29.2±3.1	< 0.0001	54.0±4.2	18.9±2.8	< 0.0001
GSH (mmol/ g. tissue)	15.9±2.6	8.4±1.6	< 0.0001	18.4±2.7	2.9±1.1	< 0.0001
MDA (nmol/ g. tissue)	15.6±3.3	31.0±4.3	< 0.0001	18.5±2.2	53.8±5.7	< 0.0001

Analysis was done by t test. Data are presented as mean ± SD. The results were considered statistically significant when the P value <0.05.
CPK: Creatine PhosphoKinase CK-MB: Creatine kinase MB
LDH: Lactate dehydrogenase CAT: Catalase GSH: Glutathione SH MDA: Malondialdhyde

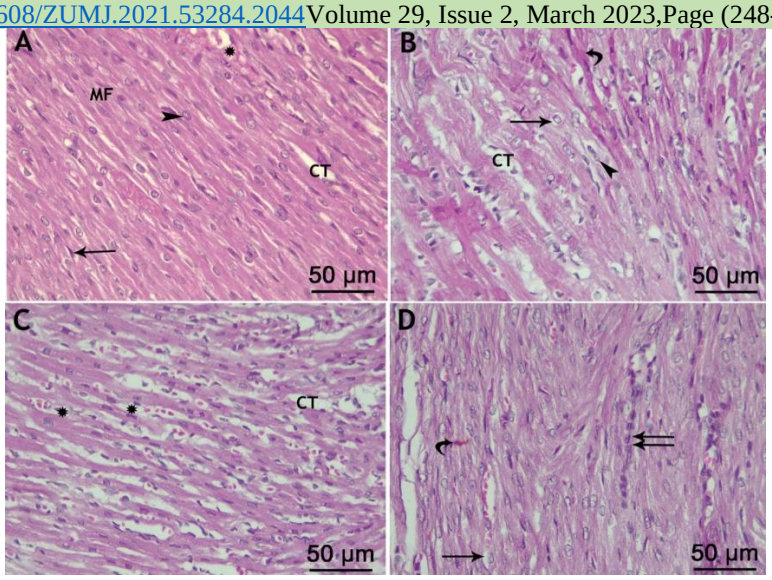

Figure1

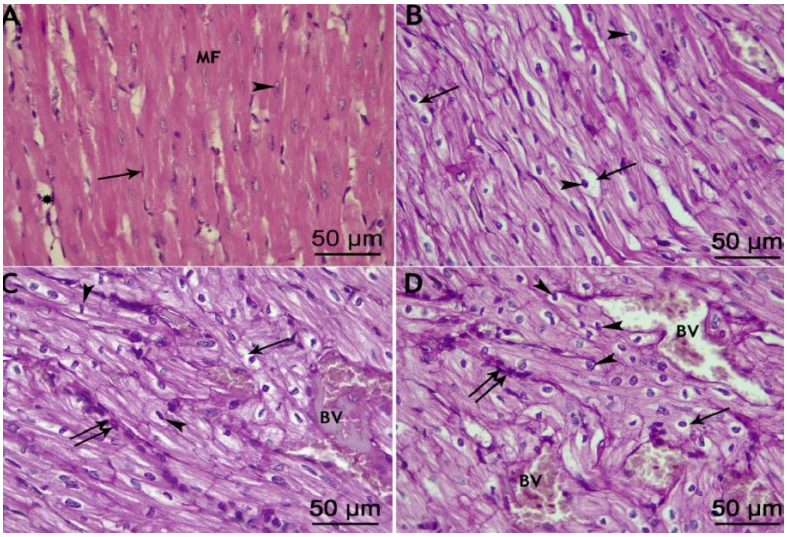

Figure 2

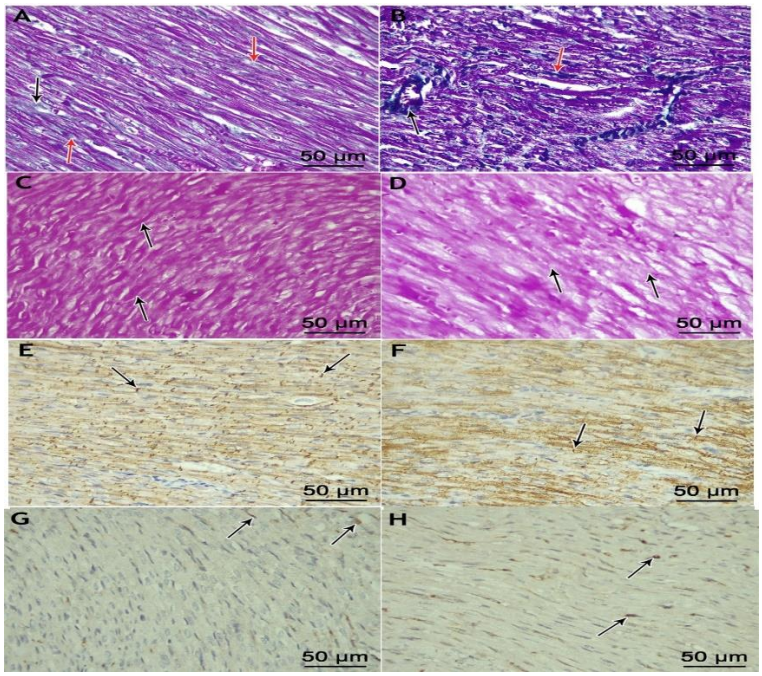

Figure 3

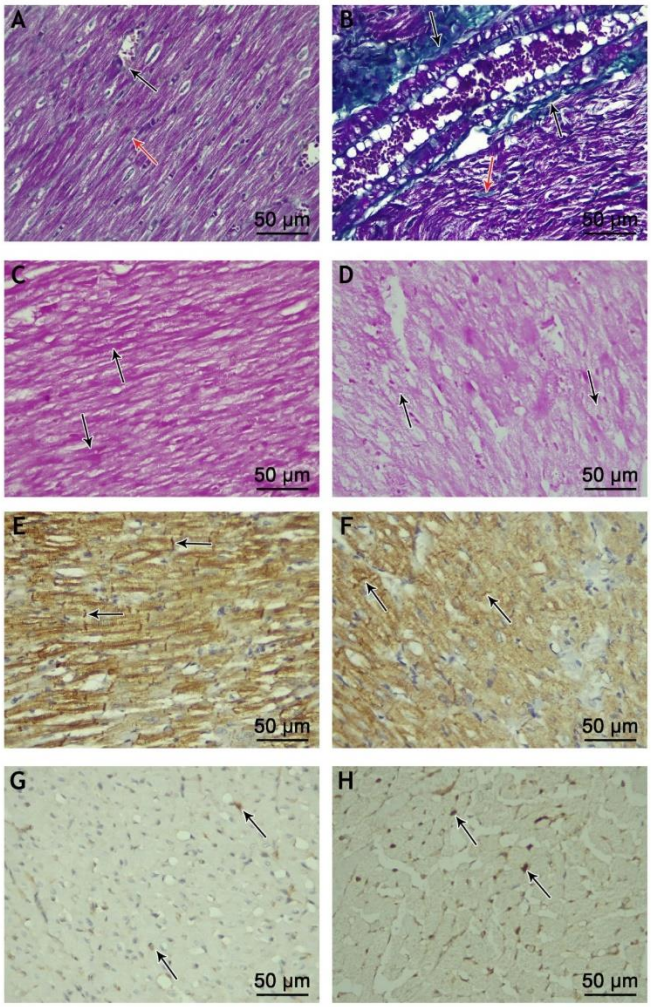

Figure 4

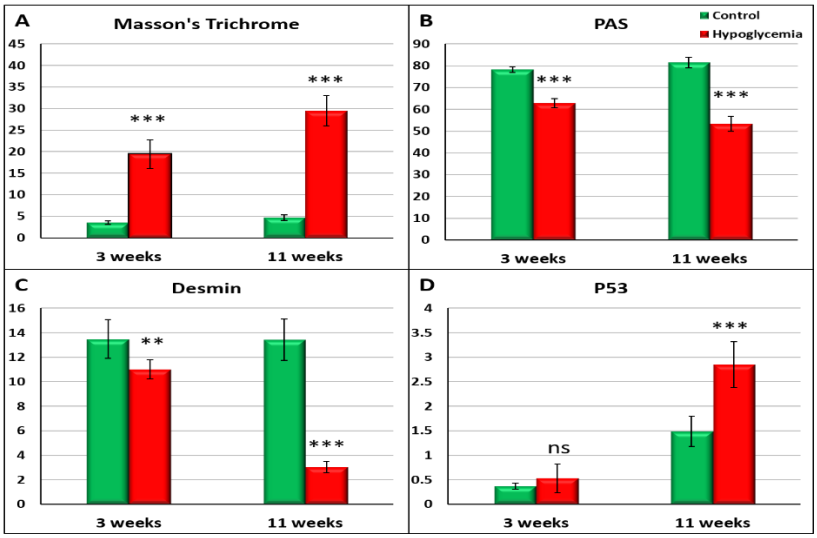

Figure 5

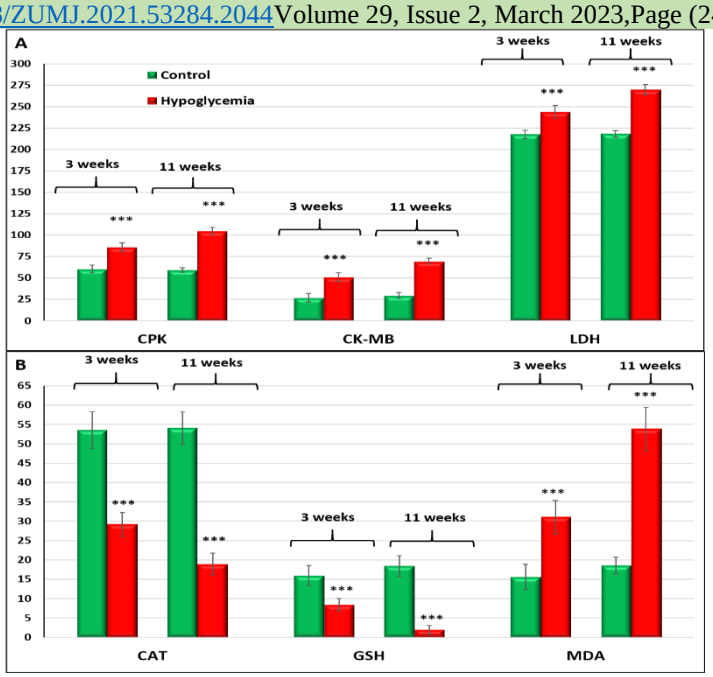

Figure 6

DISCUSSION

In the current study, an experimental work was carried out in a try to clarify the impact of intermittent hypoglycemia on the postnatal heart structure, in which a histomorphometrical cardiac study was performed in a negative control, a positive control and a hypoglycemic group of rats, from delivery to adulthood. The findings obtained showed a non-significant variations in the histological characteristics between the positive a nd negative control groups of the same age but trivial histological changes were found between different age groups. The possibility of detrimental cardiac effects of insulin-induced hypoglycemia has been increasing since the earliest use of insulin in the treatment of diabetes mellitus. Many cases of ischemic heart disease exacerbation associated with insulin therapy have been reported. Several authors have specifically identified a relationship between insulin induced hypoglycemia and acute myocardial infarction. However, since the diabetic populations are liable to both of these complications, i.e., hypoglycemia and acute myocardial infarction, it is difficult to create a causal alliance between hypoglycemia and myocardial infarction or angina pectoris aggravation **[22]**.Animal species are utilized as experimental models due to their resemblance in anatomical basis and physiological functions with humans. For example, chimpanzees and mice share about 99% and 98% of DNA with humans, respectively. As a result, animals have the affinity to be affected by many health problems affecting humans. Consequently, rats are utilized as animal models in the present work **[23].**For decades, myogenesis and cardiac development have been studied **[24]**. Laboratory animals have usually been used in these experiments to help in human consideration. The postnatal changes in heart morphology are significantly alike among all the mammalian species, but show variations in the timing of events. Such morphological changes in small laboratory animals occur more quickly than humans **[25]**.In the present study photomicrographs of the control groups reveal regularly arranged bundles of cardiac muscle fibers with single oval centrally located nuclei. This was in agreement with **[26]** who stated that in the control group of rats, the nuclei of the cardiomyocytes were single, oval, prominent and centrally located in cardiomyocytes as observed under the light microscope.

The current study showed mild degenerative changes in the hypoglycemic group sacrificed at 3 weeks postnatal, while they were more severe in the hypoglycemic group sacrificed at 11 weeks postnatal. These degenerative changes could be due to a reactive change that may be linked to the inhibitory effect on the vascular smooth muscles which induced relaxation and subsequent vasodilatation. This result is supported by **[27]** who reported that this vasodilatation and increased vascular permeability are anticipated to contribute to fluid loss from the blood. So, the vessels were engorged with blood cells with subsequent slowing down of the blood stream which would give rise to degeneration and necrosis in the cardiac tissues. In the present study the degenerative changes appeared in the form of disarrayed widely separated bundles of cardiac muscle fibers with Bizarre shaped nuclei, pyknotic nuclei, cytoplasmic vacuolation, and extravasated RBCs. Inflammatory histological changes in cardiac

tissues in the form of deformation of cardiomyocytes nuclei and disruption or disordered cardiac myofibrils caused by oxidative stress. The structural change of cardiomyocytes may have been due to the degeneration of the structural protein in mitochondria of the cytoplasm. In the current study, hypoglycemic rats verified a decline in the nuclear size of cardiomyocytes. In addition, disarrayed cardiac myofibers were observed. These findings are consistent with previous studies **[26] and [28].** Recurrent hypoglycemic attacks, being a danger stressor, were capable of triggering the above-mentioned degenerative changes in the heart. These degenerative changes were as well seen following other forms of chronic stress, including immobilization and noise stress in rats **[15].**

In addition, hypertrophy of cardiac muscle fibers found in hypoglycemic group sacrificed at 11 weeks postnatal was in harmony with **Ulrich-Lai et al. [29]** who stated that the delivered reactive oxygen species (ROS) after exposure of the tissues to hypoglycemia may elucidate both cellular hyperplasia and hypertrophy changes. These ROS cause distortion of cellular architecture with cell membrane, mitochondrial and nuclear integrity loss **[30]**.Moreover, the histological findings noticed in 11 weeks postnatal insulin treated group, in the form of apparently hypertrophied disarrayed cardiac muscle fibers, bizarre shaped nuclei and fibrosis were similar to the histopathological alterations described in cases of hypertrophic cardiomyopathy (HCM) **[31]**. In the same context, hypertrophic cardiomyopathy (HCM) in neonates was recorded in cases of hyperinsulinism **[32]**.In a trial to find an explanation of hypoglycemia induced cardiotoxicity mentioned in the current study and manifested as cardiac histopathological alterations especially manifest in 11 weeks postnatal insulin treated group, it has been recorded that, experimentally induced hypoglycemic episodes could induce sympathetic-adrenal system activation resulting in excess adrenaline release which is significant to maintain glucose supply to the brain and help the hepatic glucose synthesis. Also, hypoglycemia provokes hemodynamic alterations including increased heart rate and peripheral systolic blood pressure, a drop in the central blood pressure and in peripheral arterial resistance, widened pulse pressure with increased myocardial contractility, stroke volume, and cardiac output. This heart work overload markedly causes cardiac stress. In addition, catecholamine-mediated hypokalemia may be implicated in increased risk of cardiac arrhythmia. Additionally, a rising concern regarding long term impacts of hypoglycemia, especially as associated with inflammation and atherogenesis has been established. Furthermore, hypoglycemia could elucidate compromised endothelial integrity due to both increased C-reactive protein liberation and neutrophils and platelet mobilization and activation. These alterations may provoke intravascular coagulation, thrombosis and tissue ischemia, with the myocardium being potentially susceptible to damage **[33]** and **[34]**. Also, **[35]** documented that microvascular endothelial cell injury with subsequent vascular leakage of RBCs is an explanation of extravasated RBCs mentioned in the present study. Damaged cardiac tissue observed in the present study may be due to increased lipid peroxidation (MDA) and decreased GSH and CAT levels. In this regard **[36] and [37]** reported that the histological damage could be caused by an increase in the process of lipid peroxidation and a decrease in the activity of antioxidant enzymes resulting in cell membrane damage.The findings of the current study showed a substantial decrease in CAT activity and GSH levels in heart tissues of the hypoglycemic groups. However, MDA was increased in heart tissues of the same groups. Reduced GSH and equivalent elevation in MDA in this study may be due to oxidative stress and impairment of antioxidant defense mechanisms in hypoglycemic groups. **Krishma and Kumar [38]** credited that to the elevated utilization of antioxidant system as an attempt to detoxify the generated free radicals by hypoglycemia. The diminution in GSH levels after exposure to hypoglycemia noted in the blood serum, liver and heart tissues may be due to the reaction of GSH with free radicals resulting in the formation of GSSG **[39]**. Moreover, the accessibility of GSH can also be limited by deficiency in synthesis, improved efflux, or ineffective reduction of GSSG. In the normal condition, GSH is restored by synthesis, but in the hypoglycemic animals, normal synthesis and/or repair is disrupted due to damage to DNA and membranes **[40]**.Lipid peroxidation was believed to be an important cause of destruction and damage to cell membranes and has been shown to be a causative factor to the development of oxygen radicals-mediated tissue damage. Oxidative stress in the heart tissue was linked with a significant increase in the activity of CPK and LDH common indicators released into the blood stream from damaged hear tissue as seen in the present study **[41]**.An increase in oxidative stress leading to the overproduction of reactive oxygen species, which is frequently toxic to cells, causing damage to all components of the cells, such as proteins, DNA, and lipids **[42].** Oxidative stress is known to be a major injury mechanism concerned with the pathogenesis of disease progression including ischemic myocardial injury, and may be caused by,

or result in, inflammation **[43].**In clinical studies, a relationship between firm glycemic control, hypoglycemia and increased cardiovascular morbidity and mortality was observed **[44, 45].** Although the fundamental mechanism remains unclear, increased inflammatory cytokines and a leukocytosis are documented after hypoglycemia indicating a linkage between hypoglycemia and inflammation. However, a link between hypoglycemia and increased mortality may be present, especially as episodes of hypoglycemia might pass unrecognized and self-reporting of hypoglycemia is known to be inexact. Furthermore, a cardiac event may not be associated with an episode of hypoglycemia occurring 24 hours previously. Enhanced inflammation and increased oxidative stress may contribute to the underlying mechanism of the association of hypoglycemia with consequent cardiac death **[46, 47].**The chief intermediate filament **(IF)** in the cardiac muscle is Desmin, which accounts for about 2% of cardiac protein content. It mechanically links the z-discs to the costameres **[48].** Desmin is characterized by multiple protein-protein interactions ensuring cellular integrity, strength transmission, and biomechanical signaling. From this fundamental localization, there is no surprise that desmin knock-out will develop a multisystem disorder involving cardiac, skeletal, and smooth muscles, with the most prominent pathological processes appearing in the heart, displaying extensive cardiomyopathy accompanied by extensive fibrosis and calcification **[49]**. Constantly, up till now, as a minimum 45 mutations in the desmin gene have been identified that lead to a skeletal and cardiac myopathy called desminopathy **[50]**. Cytoskeletal IFs are typically used in animals as an indirect indicator of tissue injury **[51].**The immunohistochemical findings of the present study showed a strong decrease in the expression of desmin in intercalated discs in cardiomyocytes of hypoglycemic rats sacrificed at 3 and 11 weeks postnatal. In accordance, **Barash *et al*. [52]** discovered loss of desmin immunostaining after a short period of eccentric exercise in the rat tibialis anterior muscles. Such a large-scale change could be the result of depolymerization due to desmin phosphorylation **[53]** or the result of covalent adjustment such as ADP ribosylation **[54]**, both of which have been observed in other muscle systems. Several theories have been made in this regard co nsidering the molecular causes of the onset of heart failure. One of these, states that changes in desmin could interfere with the interface between sarcomeres and the Z band, which decreases the force transmitted to the cardiomyocyte membrane by sarcomeres **[55]**.

The present study showed that collagen distribution using Masson trichrome stain was significantly higher in the hypoglycemic groups (group II) compared to the control group (group I), suggesting that inflammatory reaction and myocardial fibrosis were significantly exacerbated in hypoglycemic rats. These findings align with those of **Nagaraja et al. [56]** who announced that hypoglycemia led to a comparative increase in the quantity and thickness of the reticular fibers that grasp the secretory cells in the cortex and medulla of the suprarenal gland. The mechanism of such increase of reticular fibers was explained by the reality that chronic stress in spite of of its type is known to augment the production of ROS that trigger the fibroblasts leading to obvious increase in the amount of collagen fibers in the adrenal gland. Also, **Yang et al. [57]** stated that excess ECM proteins, which is caused by an inequality between collagen synthesis and degradation, plays an important role in cardiac fibrosis; therefore, it is possible to use ECM protein content to evaluate the degree of myocardial fibrosis.In the present study, the reduction in the PAS staining intensity in the hypoglycemic groups is reflective of reduced glycogen content, as the glycogen is broken down to deliver glucose in order to correct hypoglycemia **[58].** These results come in agreement with the result of **[59]** who observed decreased staining affinity of PAS +ve materials in the maternal cardiac tissue of pregnant rats that exposed to 2Gy of γ-rays on day 7 or day 14 of gestation when compared to the control group.

In the present study, hypoglycemic rats showed a marked increase in the anti- P53 expression of the nuclei within the cardiac muscle fibers. These findings are consistent with previous reports **[60]** which established a major increase in total p53 protein expression in hearts of embryos exposed to 24 hours hypoglycemia compared to controls**. Fiordaliso et al. [61]** have documented that P53 glycosylation, Ang II synthesis, and ROS overproduction could be responsible for the apoptotic progression. In addition, **Frustaci et al. [62]** stated that cardiomyocyte apoptosis is controlled with blood glucose. The apoptotic cell death plays an important role in the development of heart failure. **Khaki et al. [63]** who used genetically modified mice had reported a direct causal relation between levels of apoptosis and the succession towards advanced heart failure.

Gautam et al. [60] mentioned that no clear pathways are implicated in hypoglycemia-mediated cell death in the embryonic heart. Hypoglycemia may lead to DNA damage, which activates p53 expression and triggers a response to inhibit cell proliferation, and also stated that their work has demonstrated decreased cell proliferation

in the embryonic heart as early as 6 hr after hypoglycemic exposure. Prolonged hypoglycemia may stimulate factors that trigger the apoptotic pathway, eventually causing the damaged cells to die. An increase in p53 protein expression found in response to hypoglycemia may be due to a hypoglycemia-induced increase in hypoxia inducible factor **(HIF-1) [64]**.

CONCLUSIONS

Briefly, hypoglycemia may be responsible for the histological, biochemical, histochemical and immunohistochemical alterations induced in the cardiac muscle during post natal developing of albino rats.

RECOMMENDATIONS

Special care should be exercised to avoid hypoglycemia during insulin therapy of diabetic patients with ischemic heart disease. Further studies are needed to explore the impact of recurrent episodes of hypoglycemia on different body systems.

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