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Protective effects of *Momordica charantia* extract against diabetes induced cardiac disorder in neonatal rats

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Abstract: Diabetes mellitus (DM) is one of the risk factors in the development of vascular complications. Mormodica charantia (MC) was proven to be useful in improving DM and its complications. The present study aimed to investigate the effects of MC fruit aqueous extract on cardiac histopathological changes in neonates from diabetic mothers. Neonatal rats were divided into 4 groups; group (1): neonates from non-diabetic rats considered as a control group, group (2): neonates from non-diabetic rats supplemented with 250 mg/kg. b.wt of MC fruit aqueous extract, group (3): neonates from diabetic rats without treatment, and group (4): neonates from diabetic rats supplemented with 250 mg/kg of MC fruit aqueous extract. At the end of treatment after birth, the animals were sacrificed and the heart was removed and processed for histopathological study. In the present study, neonates from diabetic mothers showed altered heart tissue biomarkers (Cardiac troponin T (cTnT), Creatine kinase (CK-MB), and Lactate dehydrogenase (LDH)). Also, an increase in Malondialdehyde (MDA) was observed in the heart tissues of the neonates of the diabetic mothers, While the level glutathione (GSH) was decreased in the heart tissue. Histopathological results showed the presence of focal cardiac fibrosis and increased cell proliferation in neonates from diabetic mothers. In conclusion, MC has a promising ameliorative effect on the cardiac dysfunction in neonate rats and the management of cardiovascular alteration associated with maternal DM.

keywords: Diabetes, Streptozotocin, *Momordica charantia,* Cardiac tissue, Neonatal Rats.

1.Introduction

Diabetes mellitus (DM) is caused by insulin deficiency, action, or a combination of them. DM is a collection of metabolic disorders that affect how carbohydrates are utilized by the body. Diabetes-related metabolic abnormalities can be brought on by low insulin production and/or insulin resistance (IR) in the target tissues. Adipose tissue and skeletal muscles are the primary targets of diabetes **(1).** T2DM is a widespread metabolic condition that increases the risk of developing diabetic cardiomyopathy and chronic cardiovascular disease (CVD), both of which can cause heart failure through several different pathways, including myocardial infarction **(2).** Pregestational diabetes mellitus (PGDM) is a serious issue for public health **(3).** The embryo, fetus, and course of pregnancy are all subjected to greater risk because of PGDM and GDM. Congenital abnormalities, particularly those of the heart, neurological system, and musculoskeletal system, are more common in PGDM patients. It may inhibit growth of the fetus when there are serious maternal problems present, particularly nephropathy. Moreover, PGDM has now been linked to several prenatal problems, including cardiomyopathy, stillbirth, and perinatal mortality **(4).** Both maternal T1DM and T2DM raise the risk of cardiovascular defects in offspring **(5).** Cardiac defects, which make up to 40% of all congenital malformations in offspring of mothers with PGDM, are the most typical malformations **(6)**. Pregnancy-related maternal diabetes is linked to fetal cardiac anatomical abnormalities, hypertrophy, and functional impairment **(7).**

Natural products, particularly of plant origin, are the main quarry for discovering promising lead candidates and are essential to the next drug development program. Plant-based remedies are the driving force behind all

existing treatments because of their simplicity of availability, low cost, and little side effects. Also, many plants provide a huge supply of bioactive compounds that have powerful pharmacological effects without any negative side effects **(8).** A vegetable plant called *Momordica charantia* L. (MC) is one of the ones used in traditional medical systems. MC is a member of the Cucurbitaceae family and is also known by the name bitter melon, bitter gourd, balsam pear **(9).** For diabetes, it is a conventional treatment. It protects functional islets while reducing islet cell necrosis and repairing damaged cells **(10).** Due to its various pharmacological benefits, including its anti-diabetic, antioxidant, anti-inflammatory, hypotriglyceridemic, and immune-stimulating properties, it has attracted considerable attention. According to some studies, the plant's leaves and fruits contained high phenolic content and showed strong antioxidant properties **(11).** Therefore, the present study investigated the effects of MC on histopathological changes in the neonatal heart tissues of diabetic rats.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) and nicotinamide (NA) were obtained from Sigma-Aldrich Company for Chemical (St. Louis, Missouri, USA). MC fruit bixa powder was purchased online from NineLife, UAE. Analytical grade materials and reagents were utilized for all other uses.

2.2. Animals

The Egyptian Institute for Serological and Vaccine Production, located in Helwan, Egypt, provided forty adult female and twenty adult male albino rats, each weighing between 150 and 200 g. The animals were kept in the animal house of the Department of Zoology, Faculty of Sciences, Mansoura University, Egypt. The bedding in the stainless-steel cages housing the rats was changed daily and included wood chips. They were housed in a climate-controlled environment with a 12-hour light/dark cycle. Before the trials started, all the rats were given two weeks to get used to the location. Throughout the study, all the rats were fed a regular meal and had unlimited access to water. During the experiment, water and a normal diet were given to each rat.

2.3. Induction of diabetes

Twenty minutes before to the intraperitoneal (i.p.) injection of STZ (55 mg/kg) Ref, female rats were given an intraperitoneal (i.p.) injection of NA (100 mg/kg) to induce experimental diabetes **(12)**. Fresh preparations of NA and STZ were made in citrate buffer (pH 4.5) **(13).** Seventy-two hours later, hyperglycemia was confirmed. Accu-Chek Blood Glucose Meters (Roche Diagnostics, Mannheim, Germany) were used to measure the animal's blood glucose level from a blood drop obtained from its tail. For the purposes of the studies, animals with fasting blood glucose levels greater than 250 mg/dl have been defined as diabetics.

2.4. Experimental design

Two female rats and one adult male were kept in separate cages, and throughout the course of the night, the diabetic and control rats mated. On the next morning, all mated females had their vaginal smears checked, and the presence of sperm or a vaginal plug was considered to mark the beginning of the pregnancy. The pregnant rats were arranged into four groups as follows: Group (1): Control pregnant rats without any treatment. Group (2): the pregnant rats were supplemented orally with 250 mg/kg of MC fruit aqueous extract daily till birth. Group (3): Diabetic pregnant rats (injected i.p. with 100 mg/kg NA then after 20 min injected i.p. with 55mg/kg of STZ before mating). Group (4): Diabetic pregnant rats were supplemented orally with 250 mg/kg of MC fruit aqueous extract daily from first day of pregnancy till birth**.**

All neonates from mothers in the various experimental groups were gathered, weighed, and anesthetized at the end of the experiment. Next, it was dissected in accordance with Mansoura University's Faculty of Science's Egyptian Bioethics Committee guidelines.

2.5. Tissue Preparation

At the end of the experimental period, neonatal rats were anesthetized, then the heart was excised carefully, wiped individually on ash-free filter paper after being repeatedly cleaned with distilled water to get rid of unnecessary material. Each heart was divided vertically into two halves, and one of them was fixed in 10% buffered formalin for

histopathological investigations. The other half was wrapped in aluminum foil and maintained at -70°C until it was used for different analyses. The heart was collected from several animals.

2.6. Biochemical analyses

Cardiac troponin T (cTnT) levels were determined quantitatively using a rat cTnT ELISA kit purchased from CUSABIO (Baltimore, Maryland, USA). Lactate dehydrogenase (LDH) activity in heart tissue was determined using a Kit from BioMed EGY- CHEM, Badr City, EGYPT. Creatine kinase CK-MB activity in heart tissue was determined using rat CK-MB ELISA kit provided by MyBiosource USA**.** Malondialdehyde (MDA) content and glutathione (GSH)**,** activities in heart tissue were determined using Biodiagnostic Co. Kit, Egypt.

2.7. Histopathological examination

Heart tissues went through a careful fixation process using a 10% buffered neutral formalin solution. They were then dehydrated using escalating ethanol grades (40, 50%, 60%, 70%, 80%, 90%, and 96%), cleared in xylene, embedded in a highly purified paraffin wax, and sectioned at a thickness of 5-7 µm. Following the sectioning process, the sections were hydrated using a descending series of ethyl alcohol and stained using hematoxylin and eosin (H&E). The stained sections were then examined and photographed using a light compound microscope to identify any histopathological alterations **(14).**

2.8. Measurement of cardiac fibrosis

To measure cardiac fibrosis, heart sections were stained with Masson's trichrome. The sections were taken and examined using a light microscope. The extent of cardiac fibrosis was subsequently determined using the observations and the VideoTesT-Morphology software (St. Petersburg, Russia). The fibrosis area (%) was expressed as the ratio of the area of collagen deposition to the total cross-section **(15)**.

2.9. Statistical analysis

GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, California, USA) was used for all statistical analyses. The results are shown as mean ± the standard error of the mean (SEM) for the (n=6). One-way analysis of variance (ANOVA) was used for statistical comparisons, and the Neuman-Keuls post-hoc test was used afterwards (**16)**. When the *P* value was ≤ 0.05, a significant difference was considered.

3. Results and Discussion

3.1. Results

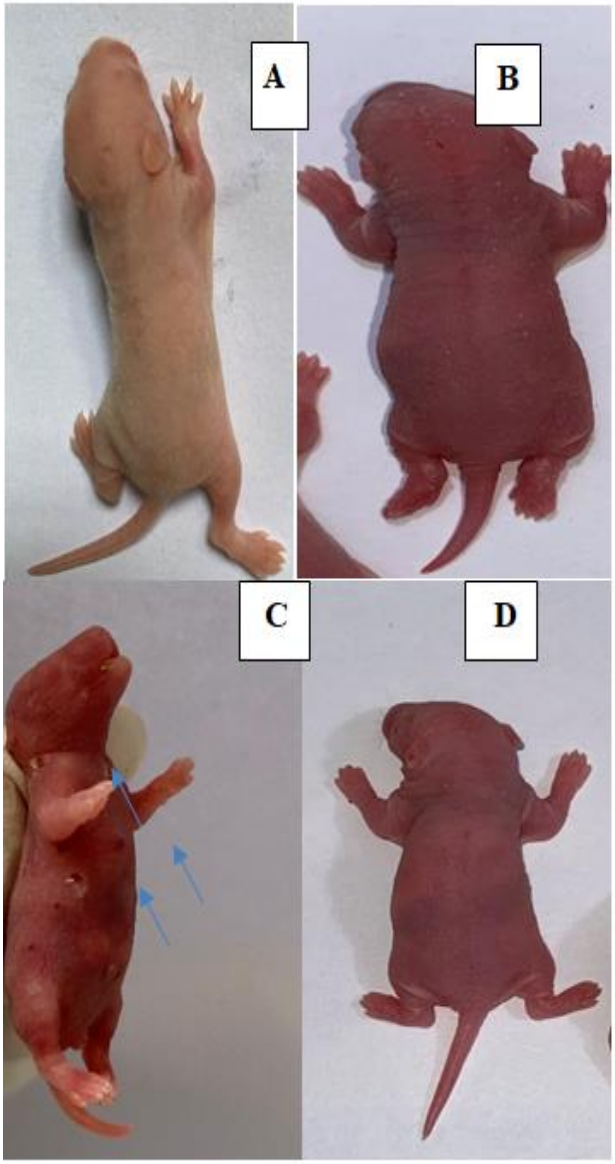

Figure (1): Neonates from different maternal groups (A) from control maternal group showing normal morphology (B) from MC maternal group showing normal morphology (C) from diabetic maternal group showing abnormal shape of morphology and many pores in the skin (blue arrows) (D) from diabetic maternal group received MC showing normal morphology.

3.1.1. Cardiac biomarkers in neonatal heart

Table (2) shows the level of cTnT, CK-MB and LDH in neonatal heart tissue of control and other diabetic maternal groups. There was an

increase in cardiac levels of cTnT, CK-MB and LDH in neonates of diabetic mother rats compared to the neonates of control rats. The neonates of pregnant diabetic rats supplemented with MC exhibited significantly reduced cardiac cTnT, CK-MB and LDH levels when compared with neonatal of pregnant diabetic rats. However, this level did not reach the level observed in newborn of the control rats.

Table (1): Maternal reproductive performance in the control and the other experimental groups

Groups Parameters	**C**	**MC**	**DM**	**DM+ MC**
Number of pregnant rats	6	6	6	6
Number of corpuses lutea	14.5	13.6	13.3	13.6
Number of alive Neonates	5.5	5.3	3	5
Weight of Neonates	4.5	4.2	4	4.4
Length of Neonates	2.9	2.45	1.9	2.3

Values are expressed as means.

Table 2: Biochemical parameters in cardiac tissue of the neonates of the control and different maternal groups

neonates' groups	**C**	**MC**	**DM**	**MC+DM**
cTnT (pg/mg)	762±4.6	744±11	1442^{a}±12	980^{ab}±19
CK-MB (pg/mg)	624±5.3	625±3.2	817^{a}±13	753^{ab}±5.7
LDH(U/g)	227±2.8	225±2.2	256^{a}±1.8	245^{ab}±2.1

Results are presented as means ±SE (6 animals per group). a, b significant changes at $P \leq 0.05$. a: significant as compared to control. b: significant as compared to DM group. MC: *Momordica charantia*. DM: Diabetes Mellitus.

3.1.2. Oxidative stress and antioxidant markers in the neonatal heart

Table (3) demonstrates the MDA and GSH concentration in the neonatal heart of control and different treated maternal groups. neonates of diabetic rats exhibited a significant increase in MDA content and decrease in GSH as compared to the neonates of control rats. While the neonates of pregnant diabetic rats supplemented orally with MC revealed significantly reduced MDA content and increased in GSH as compared to the neonates of pregnant diabetic rats. However, MDA content and GSH did not reach the content observed in the neonates of control rats.

Table 3: Cardiac malondialdehyde concentration and Cardiac glutathione reduced in the neonates of control and different maternal groups.

neonates' groups	**C**	**MC**	**DM**	**MC+DM**
MDA (nmol/g)	773±6.5	772±4.6	969^{a}±8.8	890^{ab}±8
GSH (mmol/g)	3.7±0.12	4.10.33	1.8^{a}±0.045	3^{b}±0.084

Results are presented as means ±SE (6 animals per group). a, b significant changes at $P \leq 0.05$. a: significant as compared to control. b: significant as compared to DM group. MC: *Momordica charantia*. DM: Diabetes Mellitus.

3.1.3. Hematoxylin and Eosin-stained cardiac muscles sections in neonates of diabetic rats

Histological examination of H&E-stained cardiac muscle sections from neonates of the control mother and the newborn of the maternal group that received MC showed single, oval, and centrally located nuclei of cardiomyocytes with regularly arranged cardiac myofibrils and a normal arrangement of muscle fibers with a narrow interstitial space **(fig.2).** Cardiomyocytes' nuclei in the neonates of the diabetic maternal group had deformed regarding their sizes and shapes. The cardiac myofibres in this group were also observed to be disorganized in comparison to the neonates of the control group, with hyalinization containing more eosinophilic sarcoplasm and pyknotic nuclei, a wide interstitial space, and interstitial fibroblast development. However, the neonates of the diabetic maternal group receiving MC showed fewer severe histological alterations in the cardiac tissue compared to the diabetic group in cardiac tissue with mildly disorganized muscle fibers and mild hyalinization. **(fig.3).**

3.1.4. Masson's Trichrome stained cardiac muscles sections of neonates of diabetic mother rats

Histological examination of MT-stained myocardium of the control heart of both neonates from control and MC-supplemented mothers showed no excess collagen deposition.

While the neonates of the diabetic maternal group show marked interstitial and perivascular greenish collagen deposition. Where the neonates of the diabetic maternal group received MC, it showed mild interstitial greenish collagen deposition **(Fig.4).**

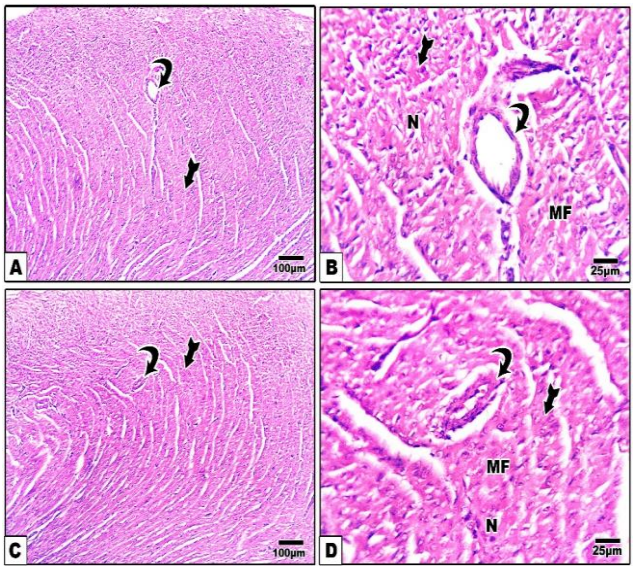

Figure 2: Photomicrographs of H&E-stained cardiac muscles sections from neonates of control group (A and B) and neonates of group received MC (C and D) showed normal arrangement of muscle fibers with narrow interstitial space. Low magnification X: 100 bar 100 and high magnification X: 400 bar 25.

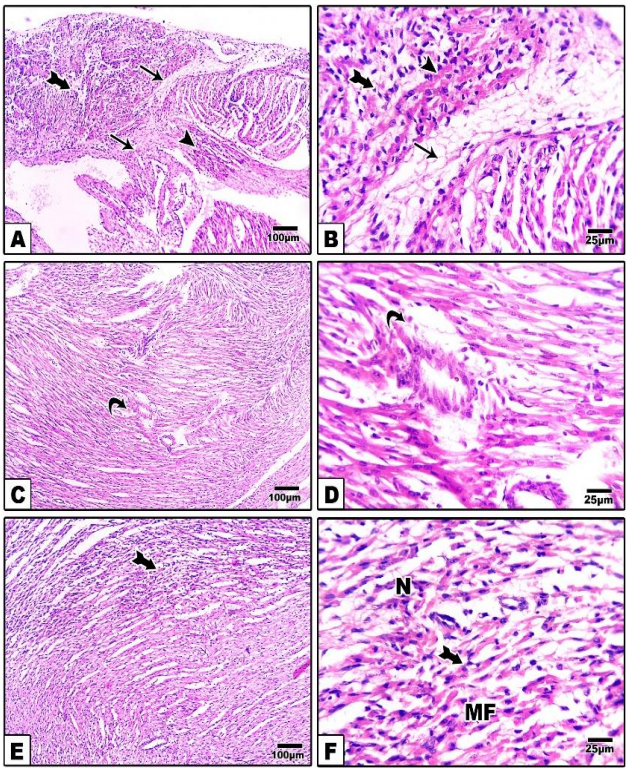

Figure 3: Photomicrographs of H&E-stained cardiac muscles sections from neonates of diabetic group (A, B, C and D) showed destruction of muscle fibers (dark black arrows), hyalinization (black arrows) with more eosinophilic sarcoplasm and pyknotic nuclei, marked perivascular (curved arrows) and interstitial (black arrowheads) proliferation of fibroblasts and collagen deposition. But in neonates from diabetic group received MC (E and F) showed shrinkage and atrophy of muscle fibers (dark black arrows). Low magnification X: 100 bar 100 and high magnification X: 400 bar 25.

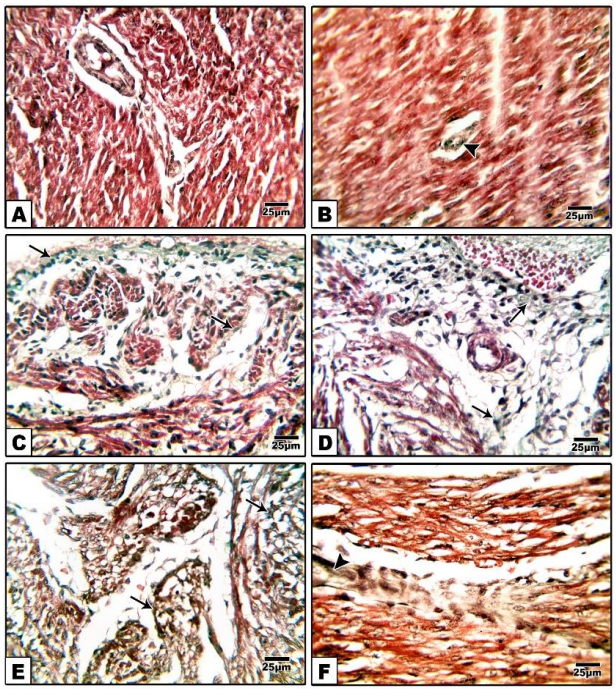

Figure 4: Photomicrographs of MT-stained cardiac muscles sections from neonates of control group(A) and neonates of group received MC (B) showed no excess collagen deposition. But neonates of diabetic group (C, D and E) showed more severe interstitial (black arrows) and perivascular (black arrowheads) greenish collagen deposition than in mother in many sections. Where neonates of the diabetic group received MC (F) showed mild interstitial (black arrows) greenish collagen deposition in few sections. High magnification X: 400 bar 25.

3.2. Discussion

An estimated 5% to 3% of pregnant women are thought to have diabetes mellitus, a common pregnancy problem. Preexisting type 1 and type 2 diabetes affect 0.27% and 0.1% of births, respectively, and the percentages are rising on a global scale. Gestational diabetes accounts for 87.5% of pregnancies complicated by diabetes; pregestational type 1 and type 2

diabetes account for the remaining 7.5% and 5% of pregnancies complicated by diabetes, respectively. Type 1 and type 2 diabetes pregestational is more serious and causes more unfavorable pregnancy outcomes. PGDM is becoming more common among reproductive females, coinciding with an increase in the global incidence of diabetes. Pregnant women with diabetes provide a difficult environment for developing embryos and fetuses **(17).**

Regarding cardiac biomarkers, the obtained results showed a significant increase in cardiac levels of cTnT, CK-MB, and LDH activity in the neonates of diabetic rats. They are sensitive biochemical parameters relating to cardiac injury, and numerous studies have reported that these markers are relatively increased during cardiomyocyte membrane and myocardial endothelial injury in DCM **(18).** PGDM is a significant risk factor for heart defects through modified maternal metabolism **(19).** Russell et al. (2009) revealed that newborns of diabetic mothers, especially those with cardiomyopathy, have higher serum markers for cardiovascular dysfunction in their cord blood **(20)**. In the present work, diabetic pregnant rats supplemented with MC showed a significant decrease in the levels of cTnT, CK-MB, and LDH activity in their neonates. Therefore, ameliorated levels introduce evidence for the protective effect of MC against DCM.

The present data revealed a significant increase in cardiac MDA contents in the neonates of diabetic rats. Furthermore, they showed a significant reduction in cardiac GSH concentration**.** Moazzen *et al.* (2014) showed that GSH levels were decreased while ROS levels were increased in the fetal heart of PGDM **(21)**. In the present study, the neonates of pregnant diabetic rats supplied with MC had a significantly reduction in MDA, as well as significantly increased in their cardiac GSH in comparison to neonates of the diabetic rats. As a result, hyperglycemia induced oxidative stress as demonstrated by increased lipid peroxidation and was significantly recovered in neonates of diabetic rats who were fed with MC. It is well recognized that flavonoids are among MC's strongest antioxidants and free radical scavengers. As the content of flavonoids increased, the antioxidant capacity gradually improved **(22).**

The histological analysis of the heart tissue in the current study showed that the cardiac tissues' structural organization was different in the newborn rats of diabetic mothers. Myocardial damage was indicated by inflammatory alterations in heart tissues. In contrast to cardiac muscles sections from control mother's neonates and neonates of mothers in the group who received MC, interstitial proliferation of fibroblasts was seen in the hyalinization with more eosinophilic sarcoplasm, pyknotic nuclei, wide interstitial space, and deformed cardiomyocyte nuclei. The MC showed a single, oval, centrally positioned cardiomyocyte nucleus with regularly spaced cardiac myofibrils, a typical configuration of muscle fibers, and a restricted interstitial space**.** Innih *et al.* (2021) demonstrated that MC preserved and repaired the heart's tissues' structural and functional integrity **(23**). It reduced the degenerative changes in the myocardium, which predicted the heart-protective or cardiovascular-beneficial effects of MC. One of the most used vegetables that naturally controls diabetes and contains polypeptide-p is MC **(24).** Insulin-like polypeptide-p, also known as p-insulin, is a hypoglycemic protein that, when administered subcutaneously, has been demonstrated to lower blood glucose levels in humans. Additionally, it was attributed to have strong antioxidant effects because of substances like glucosinolates, phenols, flavonoids, isoflavones, terpenes, and anthraquinones that could protect against alterations in myocardial damage **(25).**

The study under consideration showed marked interstitial and perivascular greenish collagen deposition in neonates from diabetic mothers. However, the newborns of diabetic mothers supplemented with MC showed recovery from the cardiac fibrosis caused by DM. Diabetes-induced myocardial fibrosis in rats was decreased by MC fruit extract. The obtained reduction in collagen expression increased free radical scavenging activity and decreased ROS, which in turn reduce oxidative stress and collagen accumulation. These mechanisms may have occurred as a result of treatment with MC extract **(26,27).** The main

finding is that MC supplemented to the diabetic mothers improved their neonates from myocardial disease and decreased the oxidative stress.

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