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Modulation of MAPK/Nrf2-HO1/Akt-eNOS/inflammasome pathways by morin in myocardial infarction in diabetic rats

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Abstract

Purpose: Diabetes is a risk factor that predisposes to atherosclerotic cardiovascular diseases. The risk of myocardial infarction in diabetes is 3-4 times higher. In diabetes, high blood glucose levels lead to vascular inflammation which accelerates atherosclerosis. Hence, we evaluate the mechanism involved in the cardioprotective action of Morin in diabetic rats.

Methods: In male Wistar rats, streptozotocin (70 mg/kg; i.p.) was administered to induce diabetes and, rats with fasting blood glucose levels >400 mg/dl were considered diabetic and included in the study. These rats were divided into five groups (n=8), i.e., Normal; Diabetic-control; Diabetes+Isoproterenol (ISO); Diabetes+ISO+Morin and, Diabetes+Morin. Morin was orally administered at the dose of 40 mg/kg for 28 days and on the 27^{th} and 28^{th} day ISO was administered to designate groups at the dose of 85mg/kg s.c., to induce myocardial infarction.

Results: Free radical generation in diabetes as well as the rush of ROS following ISO administration leads to activation of the intrinsic as well as extrinsic pathways of apoptosis. Morin significantly ($p \leq 0.05$) reduced oxidative stress (áGSH, âMDA, áSOD), cardiac injury markers (âCK-MB, âLDH), inflammation (âTNF, âIL-6) and apoptosis (âBax, áBCl_2, áCaspase-3). In addition, it also reduced serum insulin and blood glucose levels. Histopathology showed cardio-protection with morin. Akt/eNOS, Nrf2/HO-1, MAPK signalling pathways and Insulin signal transduction pathways were positively modulated by Morin pre-treatment. It also significantly modulated NLRP3 inflammasome formation.

Conclusion: Morin attenuated oxidative stress and inflammation and also modified expression of various molecular pathways to mitigate cardiomyocyte damage during ISO induced MI in diabetic rats.

Introduction

Diabetes is a group of metabolic disorders having increased blood sugar due to a decrease in insulin secretion and action, or both. Worldwide, 10.5% prevalence of diabetes mellitus (DM) aged between 20–79 years was estimated by International Diabetes Federation (IDF) in the year 2021 which is predicted to be rise by 11.3% and 12.2% by the year 2030 and 2045 respectively (Saeedi et al. 2019) Hyperglycemia acts as a risk factor for the development of cardiovascular diseases (CVDs) such as myocardial infarction (MI), atrial fibrillation (AF), heart failure (HF) and cardiomyopathy. Also, the mortality rate due to CVDs is 3 to 4 times higher in DM patients than non-DM population (Lee et al. 2019).

Several studies have shown that hyperglycemia can cause the production of advanced glycation end (AGE) products, interacts with its receptor (RAGE), and leads to oxidative stress, apoptosis, and inflammation (Daffu et al. 2013). To protect cardiac cells from oxidative stress, the nuclear factor erythroid 2- related factor/heme oxygenase-1 (Nrf2/HO-1) pathway is activated. Normally, Nrf2 resides in the cytoplasm with an inhibitory protein called keap. With increased oxidative stress, Nrf2 detaches from keap, translocates to the nucleus, and activates various antioxidants such as HO-1, superoxide dismutase (SOD), catalase, glutathione transferase, etc., which reduces the deleterious effect of reactive oxygen

species (David et al. 2017). Various other pathways are also activated during myocardial injury, among which phosphoinositide 3-kinase (PI3K)/Akt/glycogen synthase kinase (GSK)-3β pathway regulates numerous cellular functions such as cell proliferation, apoptosis, and cell survival. Also, previous studies have shown protection against heart failure in diabetes via the regulation of Nrf2/HO-1 and PI3K/Akt pathways (Duan et al. 2017; Zhang et al. 2019). Furthermore, it also activates the mitogen-activated protein kinase (MAPK) pathway, resulting in inflammation and apoptosis in the myocardium (An et al. 2020). Thus, there is a cross-talk between the signaling pathways which on activation can either protect or produce a deleterious effect in the myocardium.

The rising prevalence of diabetes-related cardiac dysfunction requires a focus towards finding a new drug moiety which can prevent the deteriorating functions of heart during high metabolic demands. Morin (3,5,7,2',4'-pentahydroxy flavone) a flavonoid present in *Prunus dulcis*, *Cudrania tricuspidata*, *Morus alba* and *Psidium guajava*. Morin acts through its antioxidant and anti-inflammatory properties and has been found effective against several disease pathologies such as liver injury, cerebral ischemia-reperfusion, cancer, cardiovascular, and renal complications (Ozdemiret al. 2020; Khamchai et al. 2020; Xu et al. 2019; Kuzu et al. 2018; Singh et al. 2021). Previous studies have documented the cardioprotective role of Morin against myocardial injury (Verma et al. 20019, 2020). Paoli and his co-workers found insulin mimicking the effect of Morin (Paoli et al. 2013). In addition, Razavi and his colleagues showed the antidiabetic potential of Morin through the inhibition of miR-29a (Razavi et al. 2019). However, the potential effect of Morin has not been assessed on cardiac dysfunction due to DM. Hence, in the present study, investigated the effect of morin on myocardial injury in streptozotocin-induced diabetic rats.

Material And Methods

Drugs and Chemicals

Morin, isoproterenol, and streptozotocin were procured from Sigma-Aldrich, USA. Enzyme linked immunosorbent assay kits for TNF (Tumor necrosis factor), Caspase-1, IL-6 (Interleukin-6), IL-10, NLRP-3 (NACHT, LPR, and PYD Domain-containing Protein-3) were purchased from CusaBio Technology LLC, USA. However, ELISA kit for AGE estimation was purchased from Korain Biotech Co. Ltd., China. Creatine Kinase-MB (CK-MB) and Lactate dehydrogenase (LDH) were obtained from Elab Sciences, Texas, USA. Insulin kit was purchased from RayBiotech, GA, USA. Primary antibodies, i.e., Akt, P-Akt, endothelial nitric oxide synthase (eNOS), GSK-3β, IKK-β, P- IKK-β, NF-κBp65, P-NF-κBp65, extracellular regulated kinase 1/2 (ERK1/2), P-ERK1/2, c-Jun N-terminal kinase (JNK), P-JNK, p38, Phospho-p38, P53, Bax, Bcl-2, caspase-3, Nrf-2, HO-1, cytochrome-C, FABP and HMGB1 were purchased from Abcam, California, USA. However, antibodies for AMPK, P-AMPK, HSP-70, HSP-27, HSP-20, Cyclin D1, and RAGE were purchased from Santa Cruz Biotechnology, Inc., California, USA. Antibody for PARP was procured from G-Biosciences, St. Louis, USA, while GAPDH antibody (raised in mice) was procured from AbbKine, China. HRP-conjugated secondary antibodies (Goat anti-Rabbit and Goat anti-Mouse) were procured from Thermo Fisher Scientific, USA, while the fluorescent (PE) labelled antibody was purchased from Abcam, California, USA. All chemicals used in the study were of Molecular Biology grade.

Experimental animals and conditions

Male Wistar albino rats were purchased from the institutional central animal facility and were acclimatized in the departmental animal house in polypropylene cages of 40x25x15 cm size. The temperature and relative humidity of the premises was maintained at 25 ± 2°C and 60 ± 5%, respectively. Light and dark cycle was maintained for 12:12 hours and animals were fed with a chow diet and water *ad libitum.*

Streptozotocin induced diabetes in rats

For induction of diabetes, rats were injected with streptozotocin (STZ), i.e., 70 mg/kg; intraperitoneally. On 3rd and 7th day after STZ administration, fasting blood glucose levels were assessed using One Touch Glucometer (SD Biosensor, India). Rats with fasting blood glucose levels 400 > mg/dl were considered diabetic and included in the study.

Study design

Total 40 animals were randomly divided into five groups (n = 8 in each group). The grouping of animals is as follows; Group 1: Normal (N); Group 2: Diabetes control (DC); Group3: Diabetes + Isoproterenol (ISO) (D + I); Group 4: Diabetes + ISO + Morin 40 mg/kg (D + I + M); Group 5: Diabetes + Morin 40 mg/kg (D + M). The test drug (Morin) was dissolved in normal saline (0.9% NaCl) and administered at the dose of 40 mg/kg, *p.o.* to the rats of groups 4 & 5 for 28 days (Verma et al. 2019, 2020). In the N, DC, and D + I groups, normal saline (1 ml/kg) was administered. On days 27th and 28th, animals of groups 3 and 4 were injected with isoproterenol at 85 mg/kg s.c., at an interval of 24 hours. On the 29th day, animals were anesthetized with pentobarbitone sodium at 60 mg/kg, intraperitoneally. Tracheostomy was done to ventilate the animal's lungs to maintain the negative pressure using a small animal respiratory system (RWD, China). Thereafter, blood was drawn from the heart and serum was separated for the estimation of serum-associated markers. Animals were euthanized with an overdose of pentobarbitone sodium, Blood was collected from cardiac puncture and the heart was excised. Isolated serum and part of the heart from each animal were snap chilled using liquid nitrogen and stored at -86°C for biochemical and molecular parameters. However, a part of the heart tissue was stored in 10% neutral formaldehyde for histopathological evaluation.

Biochemical analysis

Heart tissue was removed from − 86°C, weighed, and pulverized to make 10% homogenate in ice-chilled phosphate buffer (0.1M, pH 7.4). The homogenate was stored in 2 aliquots. The first aliquot was used for the estimation of malondialdehyde (MDA) and reduced glutathione (GSH) as per the protocol given in Ohkawa et al. and Moron et al. respectively (Ohkawa et al. 1979; Moron et al.1976). The second part of the aliquot was centrifuged at 6500 rpm; the supernatant was separated and used for the estimation of protein concentration using Bradford reagent and enzymatic activity of superoxide dismutase (SOD) using the standardized protocol of Marklund & Marklund (Marklund and Marklund 1974; Bradford 1976).

Histopathological analysis

The tissue stored in formalin was embedded in paraffin wax to make blocks following the standardized dehydration and xylene saturation process. From these blocks, thin sections (5 μm thick) were cut using microtome and taken on egg albumin precoated slides. The sections were deparaffinized and stained with hematoxylin and eosin (H&E) for histopathology. The slides were visualized under a light microscope (Dewinter technologies, Italy) by a pathologist blinded to the study groups. Structural damage was recorded from the slides and images were captured.

Estimation of serum markers

Serum separated from the blood samples was used for the estimation of cardiac injury markers (CK-MB, LDH), Insulin, and inflammatory markers (TNF, IL-6, IL-1β, NLRP3 and caspase-1) using ELISA kits according to the manufacturer's protocol.

Western blot analysis

Stored heart tissues were homogenized in RIPA buffer along with a protease inhibitor (MS-SAFE, Sigma-Aldrich, USA). Homogenate was then centrifuged at 10,000 rpm for 15 min and the protein concentration in the supernatant was estimated using Bradford's assay. A total of 40 μg of proteins were separated on 12% denatured resolving gel through polyacrylamide gel electrophoresis (PAGE) in reducing condition and transferred to a nitrocellulose membrane (0.2 μm; Bio-rad, USA). Thereafter, the membrane was blocked with BSA (3%) for 2 h at RT. The membrane was incubated overnight with the respective primary antibodies along with GAPDH antibody, followed by the particular secondary antibodies in a specific combination for 2 h at RT. After that, the bands having HRP-conjugated antibody were detected by the enhanced Chemiluminescence (ECL) method using ECL kit (Clarity western enhanced luminescence kit, Bio-rad, USA) while the fluorescence (PE)-labelled antibody was detected using MultiFluor Red channel with red epi light and Far Red (710 nm) filter under gel documentation system, FluorChem M, Protein Simple, California, USA.

Statistical analysis

Data from all groups were analyzed using one-way ANOVA followed by Bonferroni post hoc test using Sigma Plot 12.0 software. Data were expressed as mean ± SEM and *P*-value < 0.05 was considered as statistically significant.

Results

Effect on body weight, blood glucose, and serum insulin levels

Body weight and blood glucose levels of all rats were measured at the 3rd, 7th, and 35th day of the experiment. There was a significant reduction in body weight and an increase in blood glucose levels between the groups. Furthermore, a significant change in body weight and blood glucose level was there in diabetic rats when compared to their baseline values except in the normal group (N) (Fig. 1A and 1B).

In diabetic rats, there was a significant decrease in insulin levels in comparison to the normal group. Treatment with Morin for 28 days significantly improved serum insulin levels when compared to DC and D + I rats ($P < 0.05$) (Fig. 1C).

Effect on oxidant antioxidants levels and cardiac injury markers

In DC and D + I groups, there was a significant increase in the level of MDA (a marker of lipid peroxidation) and a decrease in the level of antioxidants (GSH and SOD) in comparison to the normal group ($P < 0.05$). In addition, there was an increased level of cardiac injury markers (CK-MB and LDH) in DC and D + I rats in comparison to normal rats ($P < 0.05$). This indicates the production of free radicals after the administration of STZ to the rats, which was further aggravated by ISO administration to the diabetic rats. However, treatment with Morin to diabetic rats significantly restored the oxidant-antioxidant status and decreased the level of cardiac injury markers when compared to DC and D + I rats ($P < 0.05$) as shown in Table 1.

Table 1
Effect of Morin on oxidant-antioxidant (MDA, GSH & SOD) and cardiac injury marker enzymes (LDH & CK-MB) in rat models of diabetes and myocardial injury.

Parameters	GROUPS				
	N	DC	D + I	D + I + M	D + M
MDA (nM/g tissue)	67.04 ± 2.52 [a]	78.80 ± 3.35 [a]	107.42 ± 1.40 [b]	81.39 ± 7.46 [a]	64.39 ± 6.94 [a]
GSH (µg/g tissue)	2.01 ± 0.17 [a]	1.98 ± 0.15 [a]	0.97 ± 0.08 [b]	1.68 ± 0.11 [a]	2.15 ± 0.19 [a]
SOD (U/mg tissue protein)	7.50 ± 0.58 [a]	6.32 ± 0.12 [a]	2.47 ± 0.05 [b]	4.42 ± 0.17 [c]	7.22 ± 0.65 [a]
CK-MB (U/L)	359.40 ± 18.54 [a]	485.53 ± 22.26 [b]	675.91 ± 32.28 [c]	537.13 ± 36.44 [b]	368.21 ± 15.45 [a]
LDH (U/L)	299.58 ± 24.79 [a]	418.00 ± 32.12 [a]	670.24 ± 37.57 [b]	508.18 ± 30.21 [c]	341.32 ± 25.32 [a]

Abbreviation: MDA: malondialdehyde; GSH: reduced glutathione; SOD: super oxide dismutase; LDH: lactate dehydrogenase; CK-MB: creatine kinase-MB isoenzyme. All values are expressed as mean±SEM (n=6). Error bars with different superscripts (a-c in between groups) are significantly different.

Effect on myocardial architecture

In the pathological evaluation, Normal control group rats showed well-preserved myocardium with no evidence of edema and inflammation. However, in DC rats, there was the infiltration of inflammatory cells and showed waviness in myofibrils along with endothelial swelling. Furthermore, in D+I group rats, there was marked inflammation, myonecrosis, edema, and myocardial damage. Morin treatment for 28 days in rats improved the pathological changes as there was mild inflammation and edema were observed (Fig. 2).

Effect on apoptosis

To confirm the presence of apoptosis, the level of apoptotic markers (Bax, Bcl-2, caspase-3, cytochrome-c, PARP, and p53) were measured by western blot analysis. There was an increased level of Bax, and caspase-3 and decreased levels of Bcl-2, cytochrome c, PARP, and p53 in DC and D+I groups in comparison to normal rats (P<0.05). Treatment with Morin for 28 days positively modulated the level of apoptotic markers and thus attenuated apoptosis in diabetic rats (P<0.05) (Fig. 3A-F).

Effect on MAPK and inflammatory pathway

In comparison to normal rats, there was a decreased level of ERK1/2 and P-ERK1/2 and increased levels of JNK, P-JNK, P38, and Phospho-p38 in DC and D+I groups. Increased levels of P38 and JNK further leads to the activation of the inflammatory cascade in the myocardium. Thus, there was an increased level of NF-κB and inflammatory cytokines (TNF and IL-6) and decreased levels of IKK-β in the DC and D+I groups in comparison to the normal (N) group. In contrast, treatment with Morin significantly normalized the level of MAPK signaling pathway and reduced the level of inflammatory markers (Fig. 4 A-F).

Effect on Nrf2/HO-I and Akt/eNOS pathways

Researchers have demonstrated that Morin increases the level of GSH, SOD in the myocardium of diabetic rats. It is well known that these antioxidants are the downstream pathways of Nrf2 pathway, thus assessed the expression of Nrf2/HO-1 by western blot analysis. There was significantly decreased expression of Nrf2 and increased expression of HO-1 in the diabetic rats (DC & D+I), and treatment with Morin significantly normalized their expression (Fig. 5A). Furthermore, there was significantly increased expression of Hsp70, Hsp27, P-AMPK, P-Akt/eNOS, cyclin D1, and decreased expression of GSK-3β in the Morin treatment group in comparison to DC and D+I groups (P<0.05) (Fig. 5B & 6A-D).

Effect on AGE-RAGE, FABP, HMGB1 expressions, and inflammasome pathway

In comparison to normal rats, there was increased expression of AGE-RAGE, FABP, and inflammasome pathway proteins (caspase-1, NLRP-3, and IL-1β) and decreased expression of HMGB1 in the diabetic group (Fig. 7). Pre-treatment with Morin significantly augmented HMGB1 expression and reduced AGE-RAGE, FABP, and inflammasome pathway protein expression when compared to DC and D+I groups.

Discussion

In experimental studies, Morin has shown protection against cardiovascular, cerebrovascular, diabetes, liver, and kidney diseases through antioxidant, antiapoptotic, and anti-inflammatory mechanisms. However, the effect of morin on myocardial injury in diabetic conditions is still unknown. In the present study, the effect of Morin on ISO-induced myocardial ischemia in diabetic rats was evaluated. In the results, we found that Morin reduced normalized blood glucose and serum insulin levels, strengthened the antioxidant defense system, preserved myocardial architecture, and prevented the increase in inflammation and apoptosis in the myocardium. This could be due to the regulation of multiple signaling pathways, i.e. AGE-RAGE/Nrf2/HO-1, MPAK, and GSK-3β/Akt/eNOS.

Streptozotocin is an antibiotic that is widely used to produce diabetes in experimental animals. It enters the pancreatic β cells via GLUT2 transporter. In β cells, it has a cytotoxic effect, causing DNA fragmentation, depletion of intracellular NAD+ and ATP, and mitochondrial dysfunction, which ultimately leads to necrosis of pancreatic β cells. A decrease in the number of pancreatic β cells produces a relative or absolute decrease in insulin levels resulting an increase in blood glucose levels (Szkudelski 2012). A decrease in insulin level leads to the breakdown of proteins, and fatty acids, and increased muscle wasting which reflects a decrease in body weight (Cheng et al. 2013). Similarly, in this study, we found that an increase in hypo-insulinemia, hyperglycemia, and a decrease in body weight in diabetic group animals and Morin treatment significantly reversed (but not completely) their levels.

It has been known that hyperglycemia activates the production of AGE, which then interact with RAGE and enhance the production of free radicals (Daffu et al. 2013).When diabetic rats were further subjected to isoproterenol, it leads to more production of free radicals and depletion of the antioxidant defense system. There, free radicals further react with lipids, proteins, and DNA and cause cell membrane damage. This results in lipid peroxidation and the release of cardiac injury markers (CK-MB & LDH) from the cell membrane. Previously, various studies have shown that natural antioxidants protect against myocardial injury in diabetic rats by inhibiting the AGE/RAGE pathway (Suchal et al. 2017; Thakur et al. 2021). With increased oxidative stress, the body activates the antioxidant system which prevents the generation of free radicals, lipid peroxidation, and depletion of antioxidants. During investigations, we found that towards protection from free radicals, the Nrf2 pathway was activated as shown by other researchers. On activation, Nrf2 binds to the antioxidant response elements (ARE) and increases the production of various antioxidant enzymes (David et al. 2017; Zhang et al. 2019). In line with this, we extended the analysis and found increased levels of AGE/RAGE proteins, MDA (a marker of lipid peroxidation), cardiac injury markers, and decreased antioxidants (GSH and SOD) in the DC and D+I groups rats. On the other hand, treatment with Morin reduced the AGE/RAGE levels, lipid peroxidation, LDH, and CK-MB levels and increased the antioxidant status, which could be due to the activation of Nrf2 pathway. Similar to this, previous studies have demonstrated the antioxidant role of Morin in various disease models (Ozdemir et al. 2020; Khamchai et al. 2020; Verma et al. 2020; Sang et al. 2017).

To check the effect of Morin on apoptosis in diabetic myocardium, various antiapoptotic and proapoptotic protein levels were measured. The intrinsic pathway of apoptosis involves the release of cytochrome c from mitochondria to the cytosol where it binds to Apaf-1 and causes activation of the caspase-dependent apoptotic pathway. Bcl-2 can inhibit apoptosis by preventing the release of cytochrome c from the mitochondrial membrane. Puma and Noxa are two members of the Bcl-2 family which are involved in apoptosis and have been found to be stimulated by p53. Both intrinsic and extrinsic pathways of apoptosis end at the executioner phase of apoptosis. Caspase- 3, 7, and 9 are members of the executioner phase of apoptosis which further cleaves various cytokines such as PARP leading to biochemical and morphological changes in the apoptotic cells (Elmore 2007). Previously, it has been found that Hsp 70 and 27 act at different steps of the intrinsic pathway of apoptosis and prevent apoptosis (Paul et al. 2002; Bratton and Salvesen 2010). In our study, there was a decreased level of antiapoptotic proteins (Hsp 70, 27, and Bcl-2) and increased levels of proapoptotic markers (cytochrome c, Bax, caspase-3, PARP, and p53) in DC and D+I group rats and was reverted in the Morin treatment group. In agreement with the findings, various other studies have shown the antiapoptotic potential of Morin in different tissues/ organs (Khamchai et al. 2020; Chen et al. 2017; Liu et al. 2018). Furthermore, it has been documented that Akt/GSK-3β signaling pathway regulates apoptosis and promotes cell survival, while phosphorylation of Akt has been shown to reduce apoptosis (Li et al. 2016). In line with this, we found increased levels of Akt in the Morin treatment, which might be responsible for the decreased levels of proapoptotic proteins in the treatment group. Rizvi et al. have shown the protective effect of Morin through the activation of Akt/GSK-3β pathway (Rizvi et al. 2015).

It has been well-documented that sustained oxidative stress can lead to the activation of the MAPK pathway. MAPK pathways consist of three proteins, ERK, JNK, and P38. Activation of JNK and P38 has been shown to induce inflammation in the myocardium by stimulation of transcription factors such as NF-κB/IKKβ (Wu et al. 2019). In the inactive form, NF-κB resides in the cytoplasm with the inhibitory protein IκB. In response to stressed conditions, there is phosphorylation of IκB by IKK which causes nuclear translocation of inflammatory cytokines, i.e., TNF and IL-6. HMGB1 has also been shown to increase the production of inflammatory cytokines by interacting with RAGE and toll-like receptors (Zhang et al. 2018). In this study, there was an increased level of MAPK, NF-κB/IKKβ, HMGBI, and inflammatory cytokines in the DC and D+I group rats, which were significantly reduced by Morin treatment. Recent studies have shown that Morin can act as an anti-inflammatory agent by inhibition of inflammatory pathways (Khamchai et al. 2020; Chen et al. 2017).

Recently, the role of inflammasomes has been extensively studied in myocardial ischemic injury, Alzheimer's disease, and diabetes (Guo et al.2015). NLRP3 inflammasome consists of NLRP3, cysteine protease pro-caspase-1, and the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). In response to oxidative stress, NLRP3 forms a complex with ASC which results in caspase-1 activation. Activated caspase-1 induces the cleavage of IL-1β and IL-18 to their active form, which causes inflammation in the tissue. Activation of caspase-1 also leads to inflammation-related cell death known as pyroptosis (Takahashi 2019). In the present study, there was an increased level of inflammasomes (NLRP3, IL-1β, and caspase-1) in the DC and D+I groups and treatment

with Morin reduced levels of inflammasome proteins. Li et al. have shown that morin protects against *Listeria monocytogenes* induced infection by inhibition of the inflammasome pathway (Li et al. 2020).

Conclusions

The present mechanistic study demonstrated that Morin attenuated oxidative injury, apoptosis, and inflammation in myocardial injury in diabetic rats. The mechanism responsible for this cardio-protection could be due to cross-talk between MAPK/Akt/GSK3β, Nrf2/HO-1, and inflammasome pathways.

Declarations

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Ethical approval

All experiments were carried out after taking approval from the Institutional Ethics Committee of All India Institute of Medical Sciences, New Delhi, India (Registration No. 10/GO/ReBi/S/99/CPCSEA; File No. 03/IAEC-1/2017).

Competing Interest

The authors declare no conflicts of interest for this publication. The expressed views in this article are of the authors alone.

Authors' Contribution

Conceptualization, V.K.V., S.M., J.B. and D.S.A.; methodology, V.K.V. AND S.M.; validation, V.K.V., S.M., J.B. and D.S.A.; formal analysis, V.K.V.; investigation, V.K.V., S.M., A.K.S. and V.P.; resources, V.K.V., J.B. and D.S.A.; data curation, V.K.V., A.K.S. and J.B.; writing—original draft preparation, V.K.V., S.M.; writing—review and editing, A.K.S., P.M. and J.B.; visualization, J.B.; supervision, J.B. and D.S.A.; project administration, V.K.V. and D.S.A.; funding acquisition, V.K.V. and D.S.A. All authors have reviewed and agreed to the published version of the manuscript.

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Availability of data and materials

Data is available with the author and available on request.

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Figures

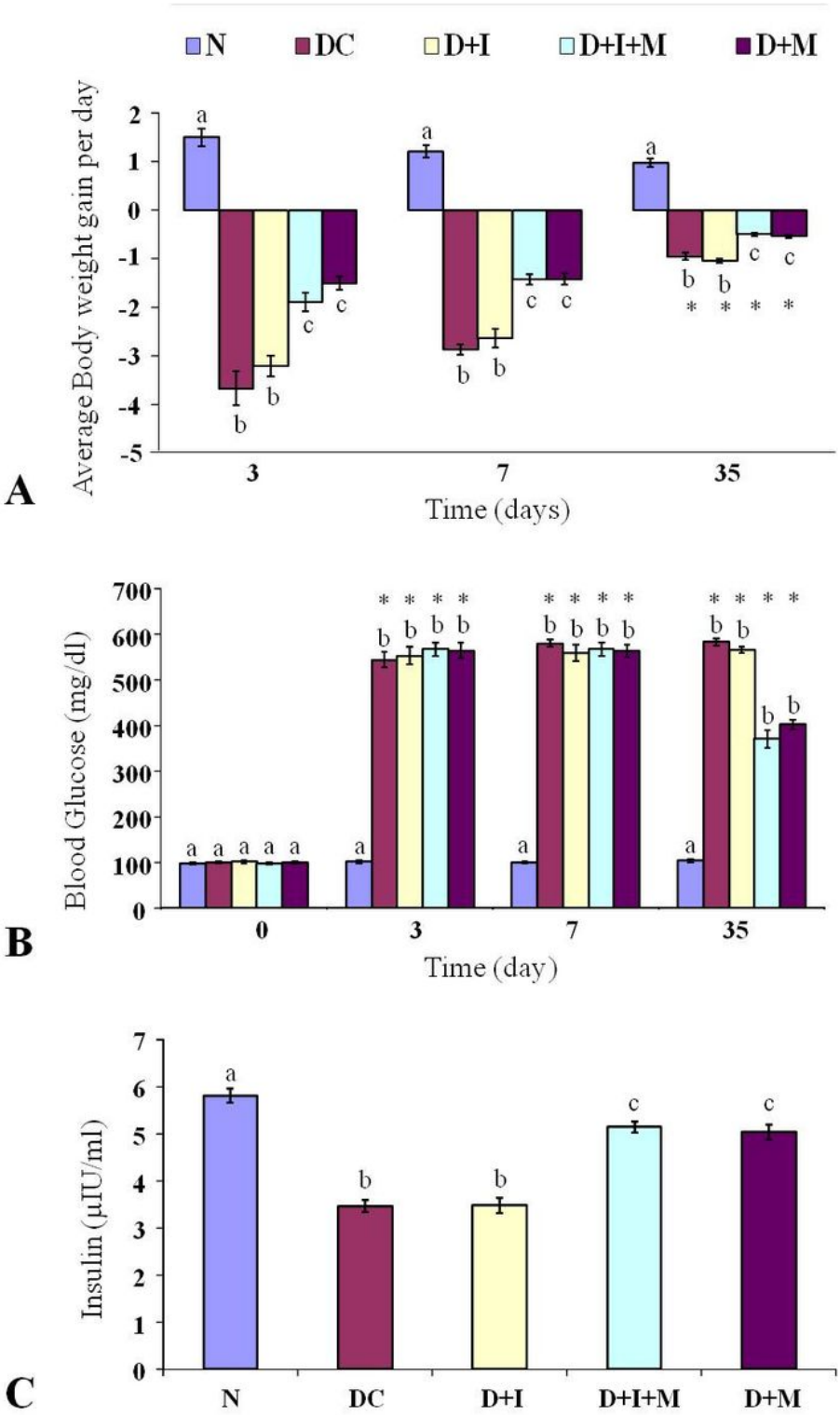

Figure 1

Effect of Morin on (A) average weight gain per day in grams, (B) blood glucose levels, and (C) serum insulin levels. All values are expressed as mean±SEM (n=6). Error bars with different superscripts (“a-c” in between groups; “*” within group at different time interval) are shown as significantly different.

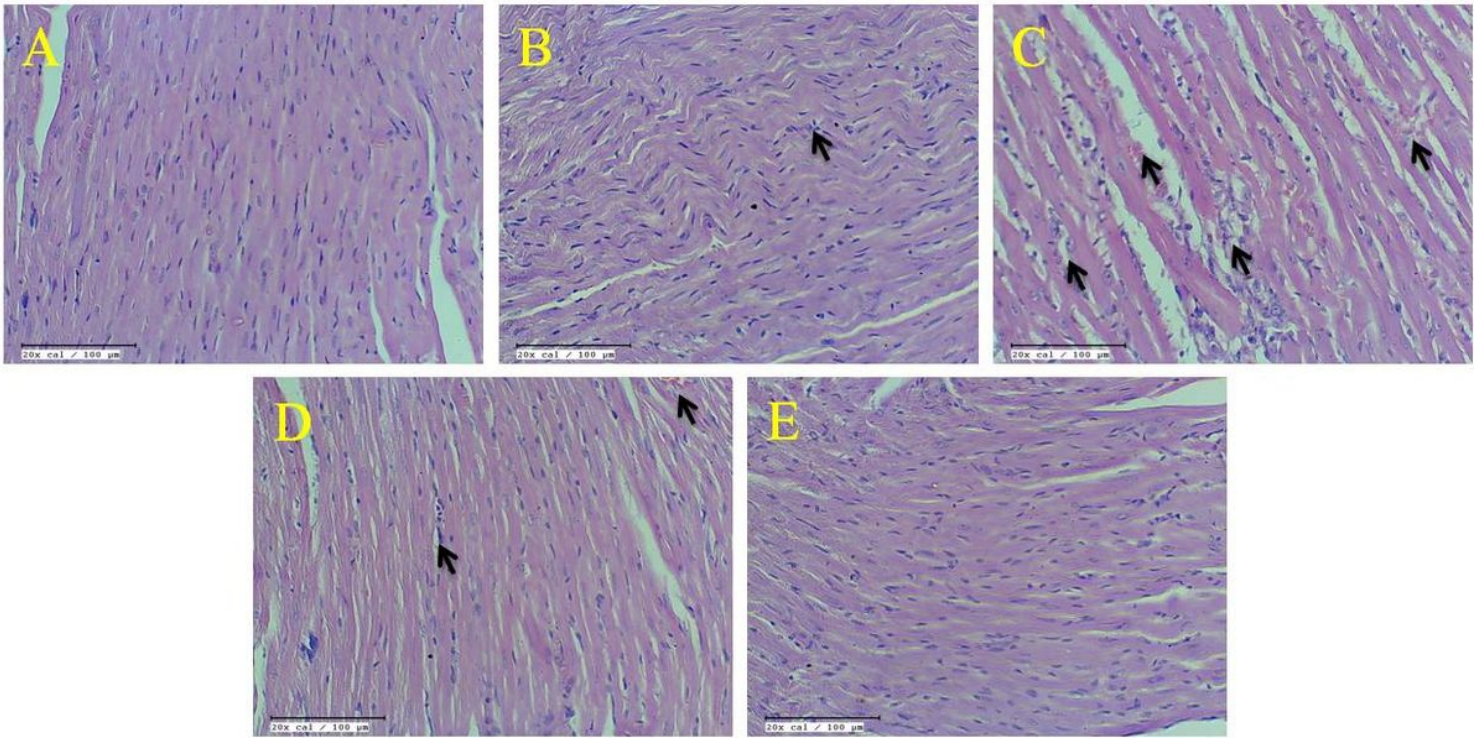

Figure 2

Effect of Morin on histopathological changes in rat models of diabetes and myocardial injury (200x; scale bar 100μm; n=3). Arrow represents the myocardial damage as neutrophil infiltration, myocardial membrane damage, and necrosis.

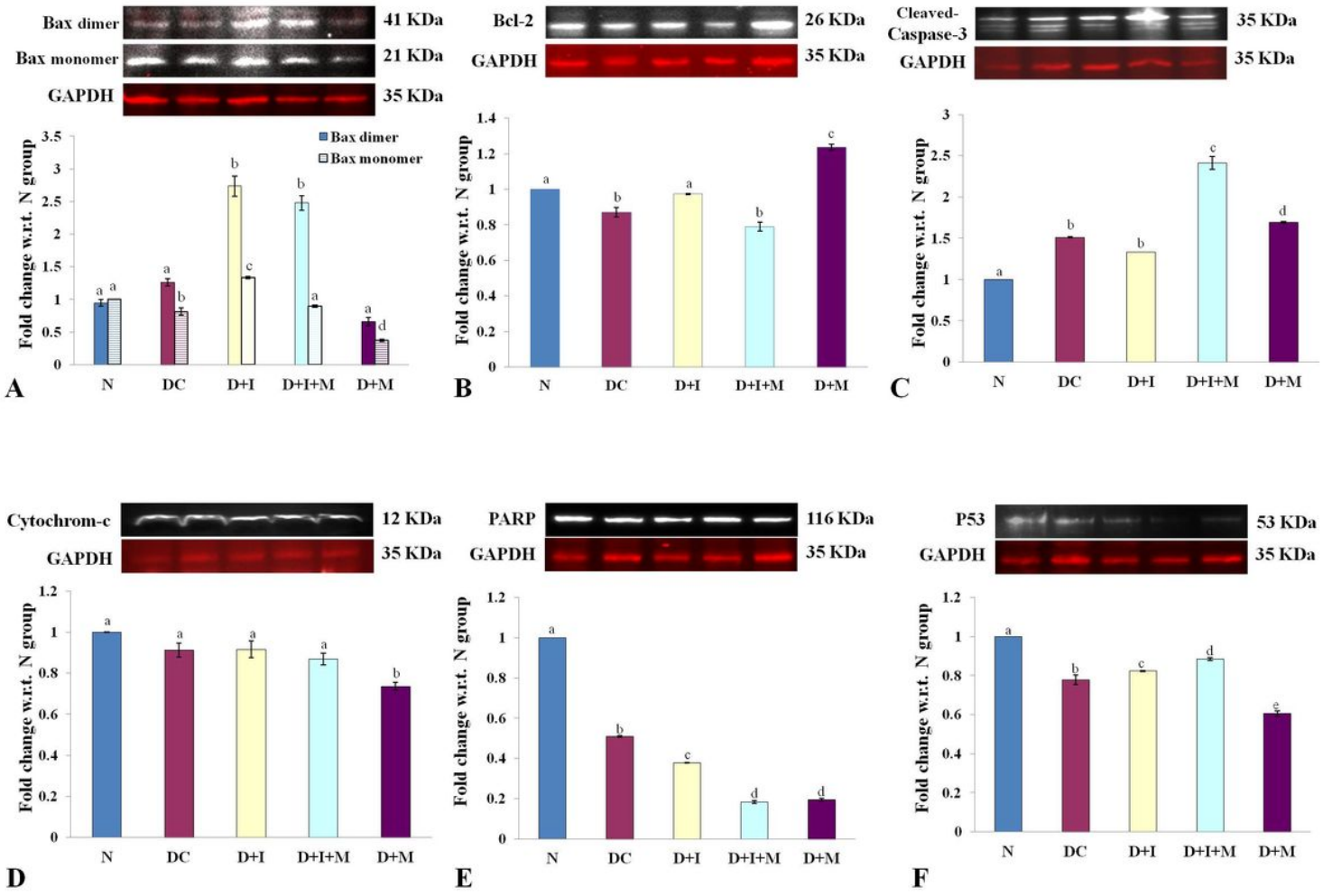

Figure 3

Effect of Morin on apoptotic pathway proteins Bax (A), Bcl-2 (B), cleaved-caspase-3 (C), cytochrome-c (D), PARP (E) and p53 (F) in rat models of diabetes and myocardial injury. Bars represent the relative intensity of respective protein normalized with GAPDH protein intensity. Error bars with different superscripts (a-e in between groups) are significantly different.

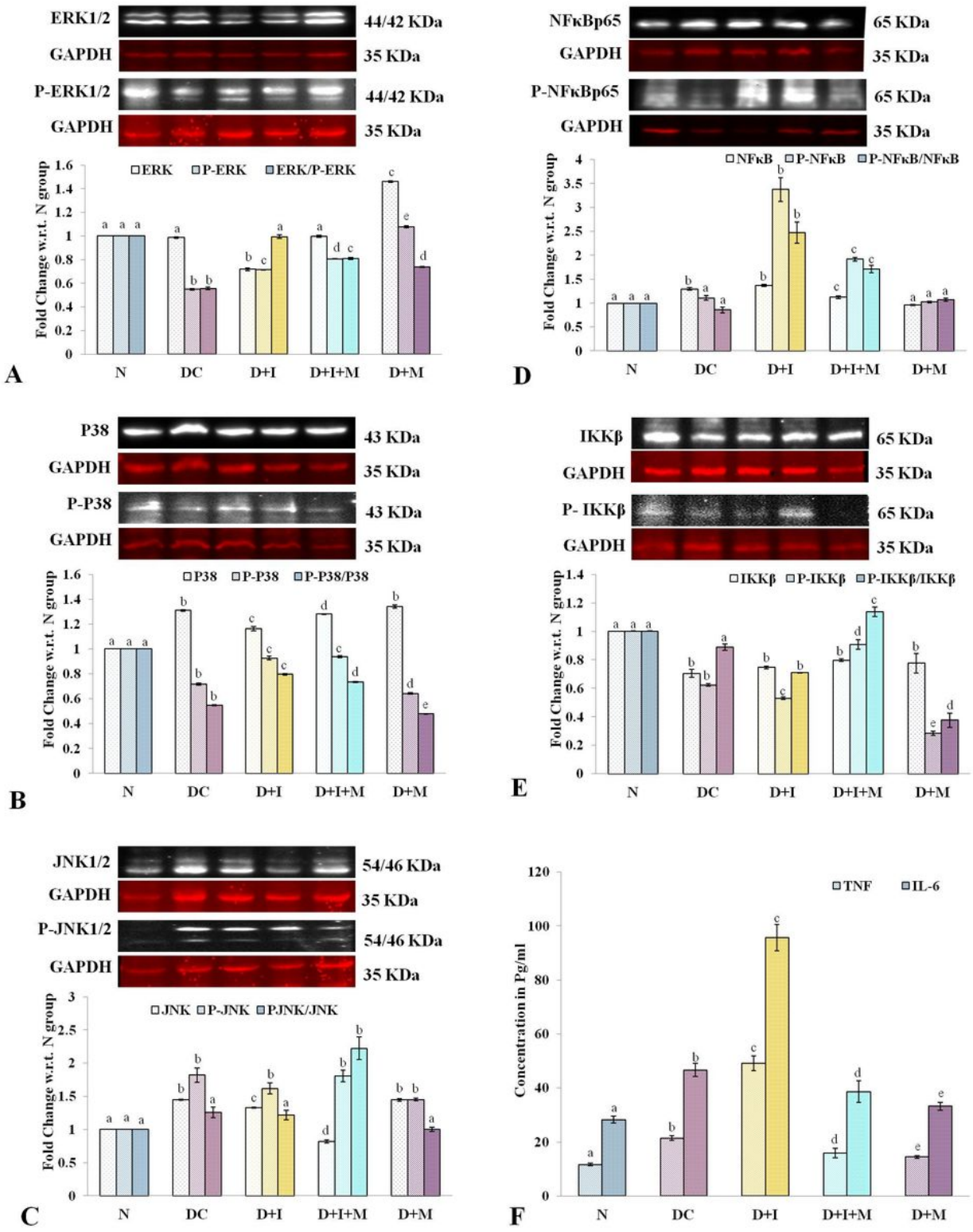

Figure 4

Effect of Morin on MAPK [ERK/ P-ERK (A); P38/ P-P38 (B); JNK/ P-JNK (C)] and inflammatory pathway [NF-κBp65/ P- NF-κBp65 (D); IKKβ/ P-IKKβ (E); TNF/ IL-6 (F)] proteins in rat models of diabetes and myocardial injury. Bars represent the relative intensity of respective protein normalized with GAPDH protein intensity. Error bars with different superscripts (a-e in between groups) are significantly different.

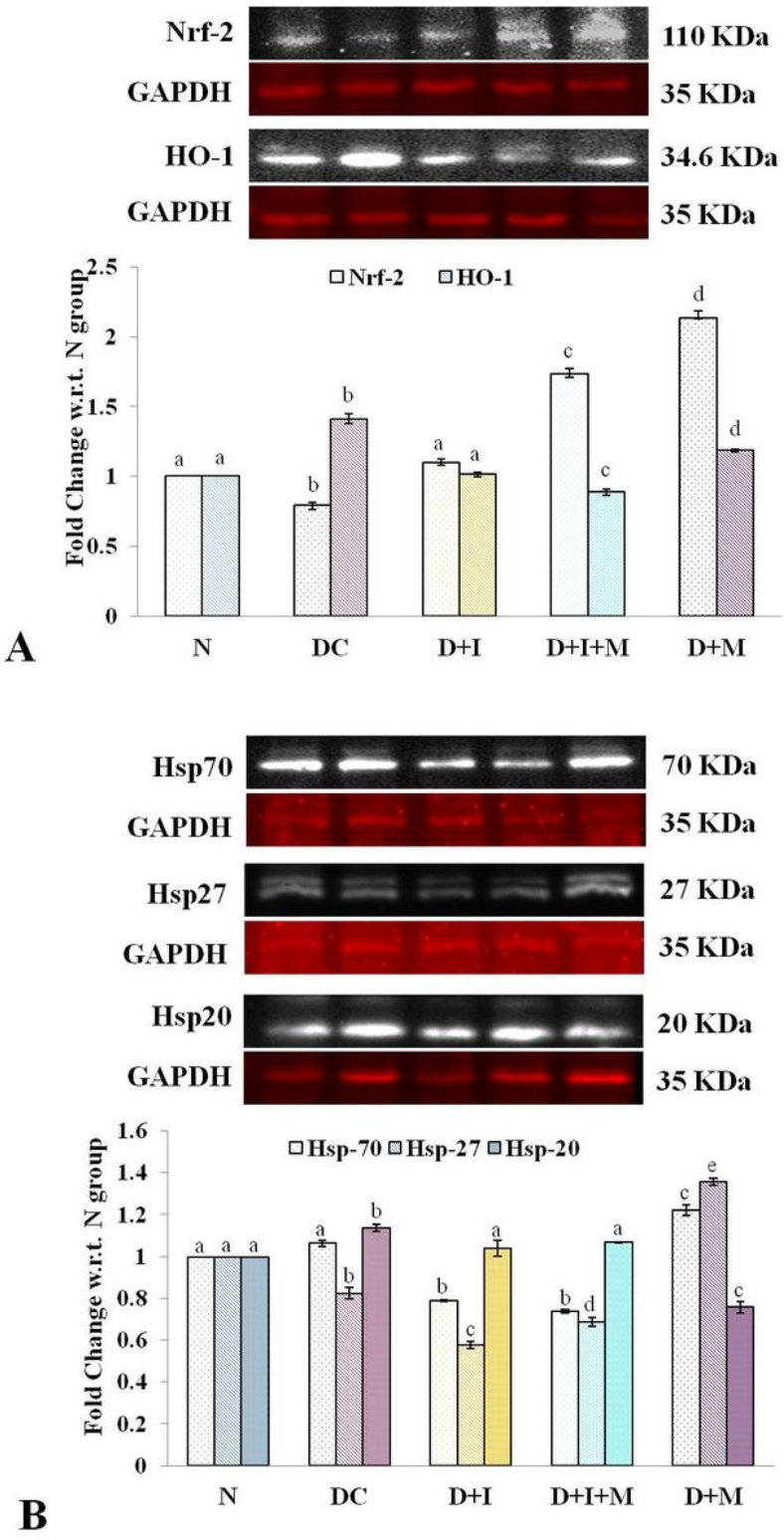

Figure 5

Effect of Morin on (A) Nrf-2/ HO-1 and (B) Heat shock proteins (70/ 27/ 20) in rat models of diabetes and myocardial injury. Bars represent the relative intensity of respective protein normalized with GAPDH protein intensity. Error bars with different superscripts (a-e in groups) are significantly different.

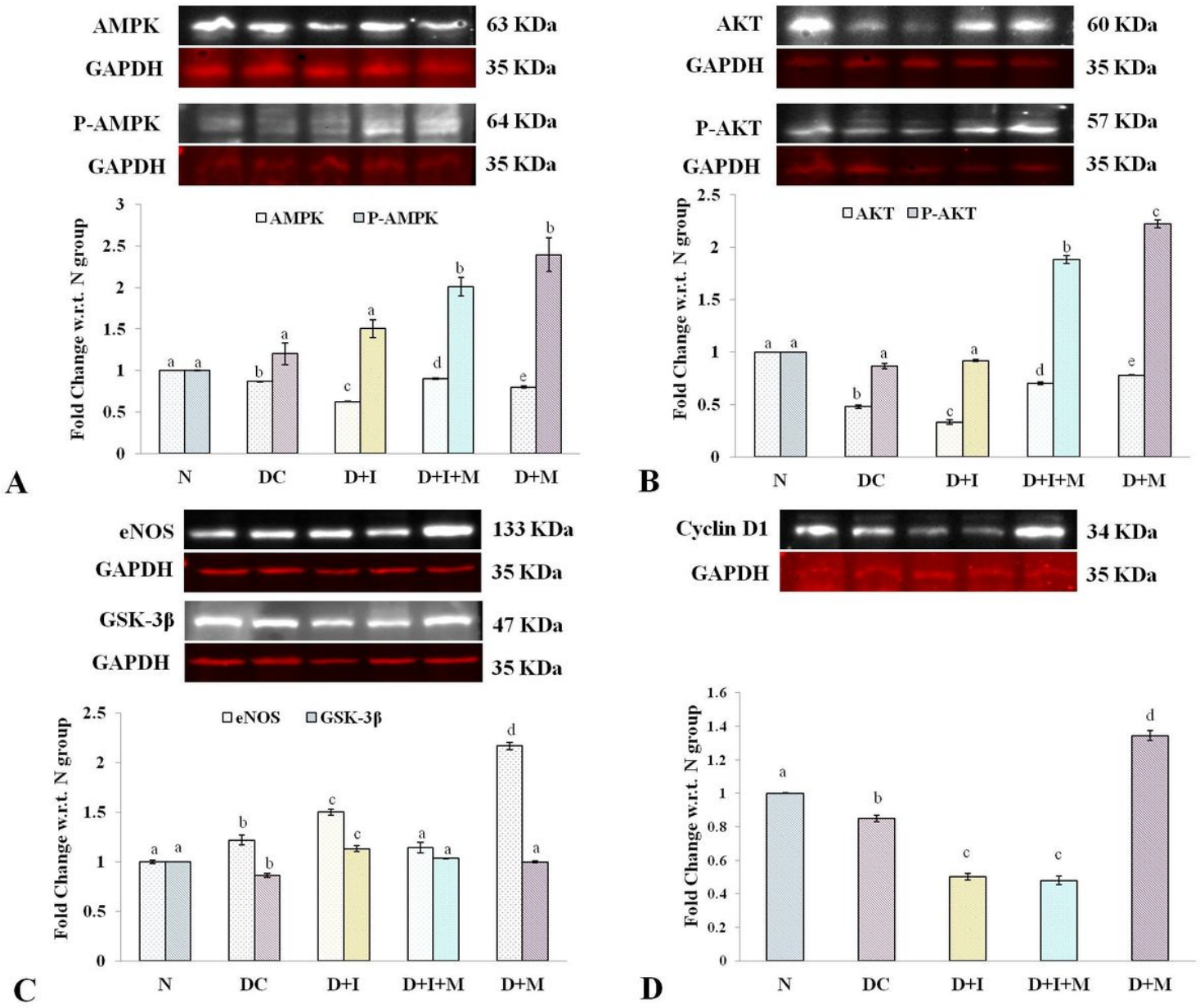

Figure 6

Effect of Morin on Akt/eNOS signaling pathway proteins [AMPK/ P-AMPK (A); AKT/ P-AKT (B); eNOS/ GSK-3β (C) and Cyclin D1 (D)] in rat models of diabetes and myocardial injury. Bars represent the relative intensity of respective protein normalized with GAPDH protein intensity. Error bars with different superscripts (a-e in between groups) are significantly different.

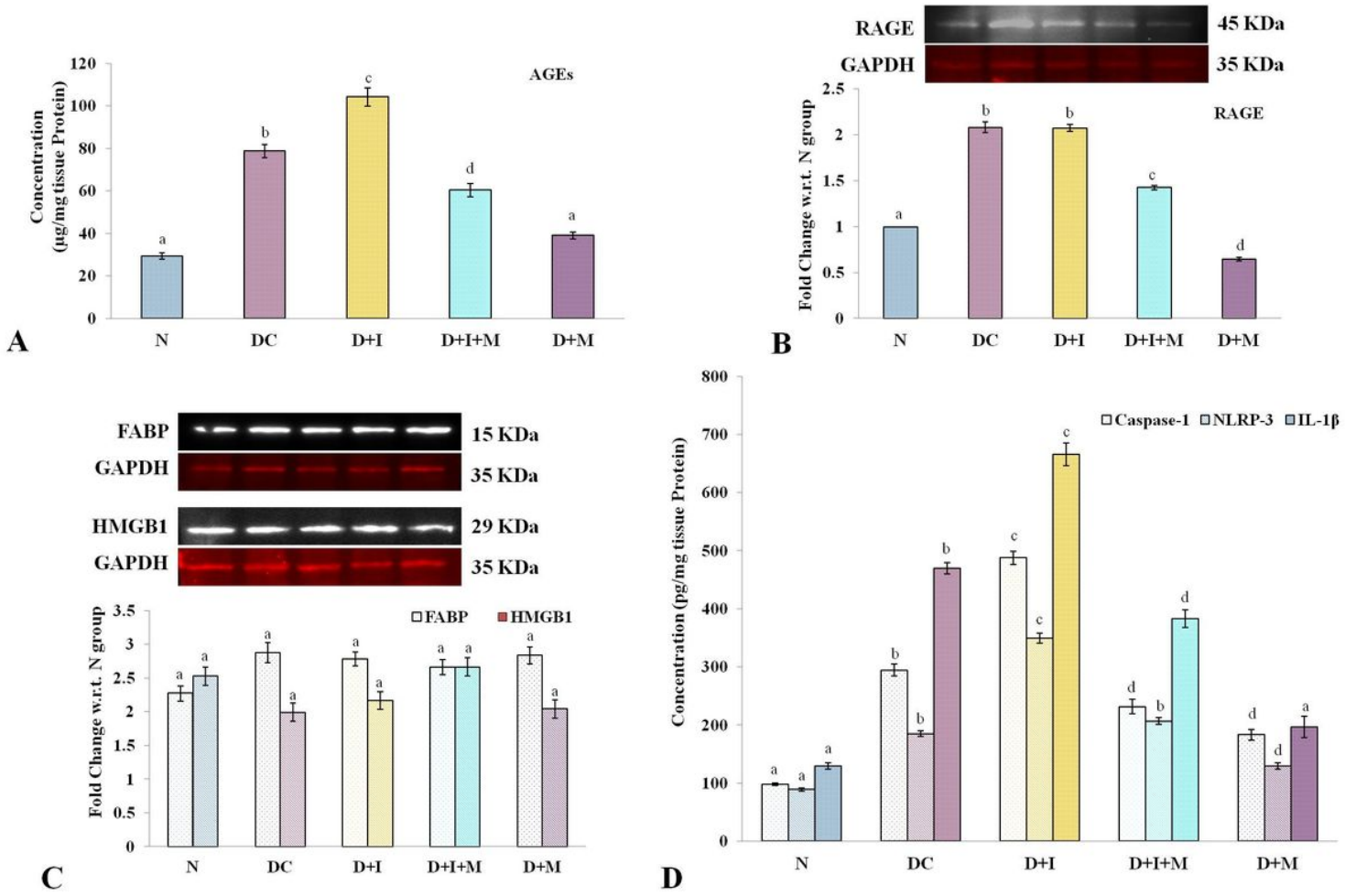

Figure 7

Effect of Morin on (A) AGEs, (B) RAGE, (C) FABP & HMGB1, and (D) inflammasome pathway proteins (caspase-1, NLRP3 & IL-1β) in rat models of diabetes and myocardial injury. Bars represent the relative intensity of respective protein normalized with GAPDH protein intensity. Error bars with different superscripts (a-e in between groups) are significantly different.

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