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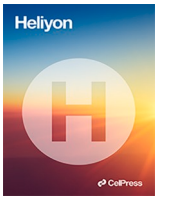

Chrysin restores the cardioprotective effect of ischemic preconditioning in diabetes-challenged rat heart

Geetanjali Singh [a,**], Vibhav Varshney [a], Ahsas Goyal [a,***], Nemat Ali [b], Muzaffar Iqbal [c], Ishnoor Kaur [d], Celia Vargas-De-La-Cruz [e,f], Tapan Behl [g,*]

[a] *Division of Pharmacology, Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, India*
[b] *Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[c] *Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[d] *College of Medical, Veterinary and Life Sciences, University of Glassgow, Scotland, United Kingdom*
[e] *Department of Pharmacology, Bromatology and Toxicology, Faculty of Pharmacy and Biochemistry, Universidad Nacional Mayor de San Marcos, Lima, Peru*
[f] *E-Health Research Center, Universidad de Ciencias y Humanidades, Lima, Peru*
[g] *Amity School of Pharmaceutical Sciences, Amity University, Punjab, India*

A R T I C L E I N F O

Keywords:
Ischemia reperfusion injury
PI3K
eNOS
Oxidative stress
Chrysin

A B S T R A C T

Background: Ischemic preconditioning (IPC) is the utmost capable design to achieve protection over ischemia-reperfusion injury (I/R), but this phenomenon gets attenuated during various pathological conditions like diabetes. Chrysin exhibits cardioprotection in various experiments however, its therapeutic potential on IPC-mediated cardioprotection via PI3K-Akt-eNOS pathway in streptozotocin (STZ) triggered diabetes-challenged rat heart is yet to be assessed. For that reason, the experiment has been planned to investigate chrysin's effect on the cardioprotective action of IPC involving the PI3K-Akt-eNOS cascade in rat hearts challenged to diabetes.
Methods: The project was accomplished through means of absorbance studies for biochemical parameters, infarct size measurement (TTC stain) and coronary flow.
Results: The findings of the present study revealed that STZ drastically augmented the serum glucose level and the chrysin significantly reversed the IPC-stimulated increased coronary flow, nitrite release, and reduced LDH (lactate dehydrogenase), CK-MB (creatine kinase) activities as well as infarct size in diabetes-induced rat heart. Furthermore, chrysin also reversed the IPC-induced reduction in oxidative stress in an isolated Langendorff`s perfused diabetic rat heart. Moreover, four episodes of preconditioning by either PI3K or eNOS inhibitor in chrysin-pretreated diabetic rat hearts significantly abolished the protective effect of chrysin.
Conclusion: Consequently, these observations suggested that chrysin increases the therapeutic efficiency of IPC in mitigating I/R injury via PI3K-Akt-eNOS signalling in diabetes-challenged rat hearts. Hence, chrysin could be a potential alternative option to IPC in diabetic rat hearts.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: geetanjali050@gmal.com (G. Singh), ahsasgoyal1990@gmail.com (A. Goyal), tapanbehl31@gmail.com (T. Behl).

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1. Introduction

In the present-day context, ischemic heart disease (IHD) stands out as a significant contributor to mortality [1]. The prevalence of IHD has increased with other comorbid situations such as ageing, diabetes, and hypertension [2–5]. It has been suggested that reperfusion is essential to maintain the usual functioning of the heart to improve the prognosis of the patients [6–9] However, rapid reperfusion threatens itself and causes irreversible myocardial injury [10]. Further, it is also documented that IPC i.e. controlled

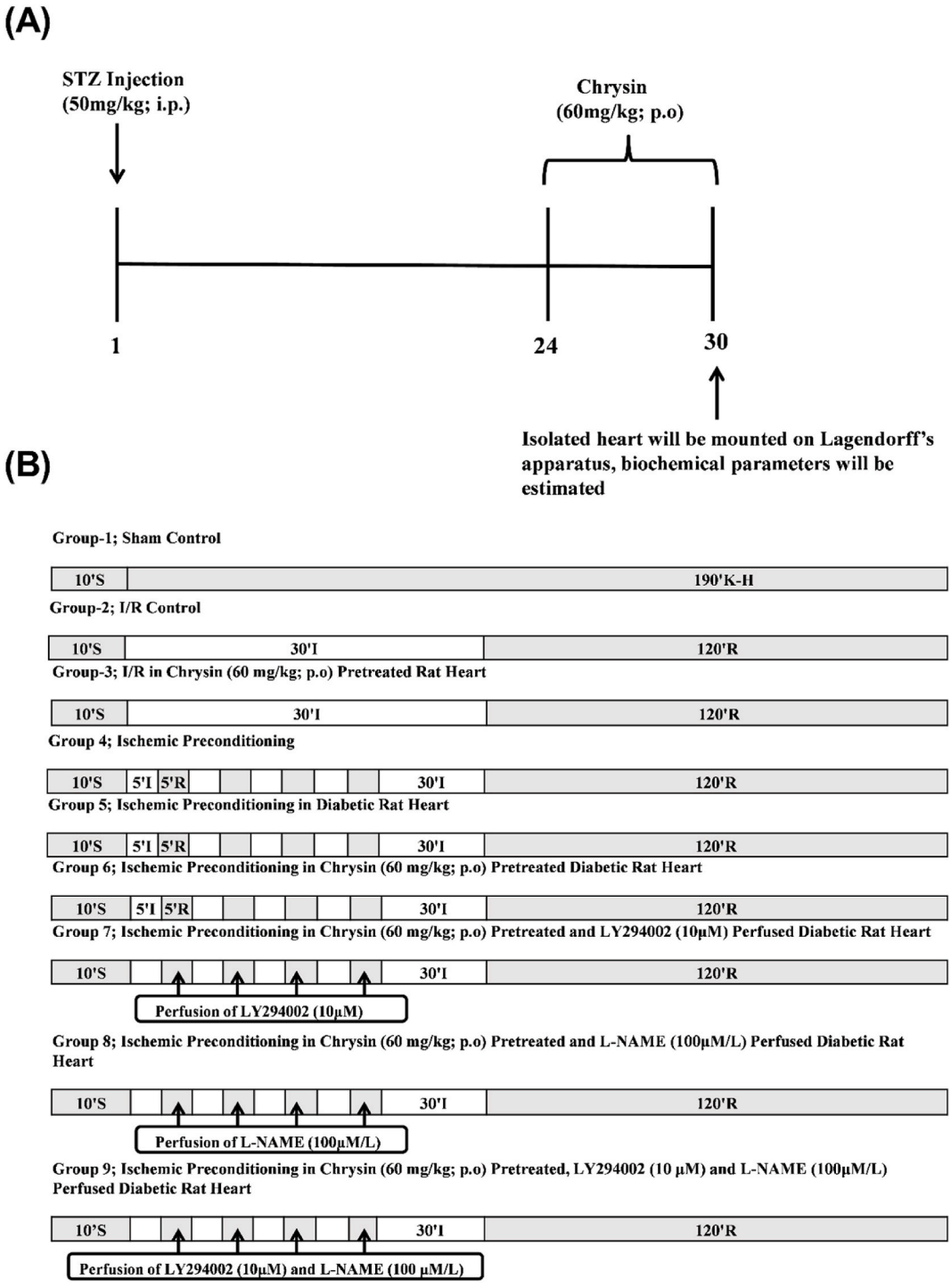

Fig-1. Diagrammatic representation of detailed experimental schedule (A) and protocol mounted on Langendorff's apparatus (B).

reperfusion through transient phases of ischemia as well as reperfusion alterations prior to an ischemic event mitigates I/R injury [11]. On the other hand, IPC's cardiac-protective effects were greatly diminished in various conditions like menopause, hyperlipidemia, ageing, hypertension, and diabetes [3,9,12–14].

Diabetes Mellitus (DM) represents a rapidly rising metabolic ailment linked to inflammation, from a diversity of complications, and is the major reason for morbidity and mortality, which significantly compromise the various protective mechanisms of the heart [15,16]. It has been suggested that increasing blood glucose level increase the prevalence rate of IHD [17,18]. In addition, it was shown that IPC was suppressed in a diabetic animal model [9,19–21] while some other laboratories are conflicted in this regard [22–24]. Consequently, the exact mechanism implicated in the suppression of this endogenous defensive system in diabetic rats is still a matter of considerable controversy.

A galaxy of preclinical studies has reported the contribution of the PI3K-Akt-eNOS route in cardioprotection by reducing oxidative stress [25,26]. However, this pathway gets downregulated in diabetic conditions [25,26]. Consequently, it is possible that the PI3K-Akt-eNOS signalling serves a function in the myocardial protection induced by IPC in diabetes.

Chrysin is a bioactive dietary phytochemical available in a variety of vegetables, fruits, medicinal plants propolis, honey, and mushrooms [27–29]. Chrysin possesses neuroprotective, antiviral, antibacterial, hepatoprotective, antioxidant, anti-inflammatory, anti-apoptotic, along with anti-cancer activities [30]. Further, chrysin reduces blood cholesterol and glucose levels, which are effective parameters for maintaining normal cardiac functions [31–33]. However, it has been suggested that chrysin induces cardioprotection through its antioxidant properties [34]. Moreover, it has been documented that chrysin can activate PI3K/AKT and also modulate endothelial nitric oxide (eNOS) in experimental animals [34–37].

Therefore, this study intended to assess chrysin's impact on the cardioprotective action of IPC through the PI3K-Akt-eNOS pathway in diabetes-challenged rat hearts.

2. Materials and procedures

2.1. Chemicals & reagents

Sigma (in St. Louis, MO, USA) provided chrysin, L-NAME, LY294002, Streptozotocin, as well as 2,3,5-Triphenyltetrazolium chloride. LY294002 along with l-NAME were incorporated into freshly formulated Krebs-Henseleit (K–H) physiological salt solution. The remaining chemicals and reagents were collected from nearby suppliers (from Himedia Laboratories Pvt Ltd. in Mumbai as well as Merck Pvt Ltd. in New Delhi). All the reagents as well as chemicals met the standards of analytical quality.

2.2. Experimental animals

Wistar albino were grouped at random and accommodated in polyacrylic enclosures, which were lined with husk, in accordance with standard environmental factors (45–55 % of humidity, 24 ± 2 °C temperature as well as 12:12 h rounds of daylight and darkness) were sourced from the animal facility of GLA University's Institute of Pharmaceutical Research, Mathura. Each animal was given unrestricted access to feed pellets (Lipton India, Ltd., Mumbai) as well as water. Animals in a state of fasting (16–18 h of the derivation of food) were used for the present experimental purpose. All procedures and protocols were carried out in compliance with the Institutional Animal Ethical Committee's (IAEC) approval (GLAIPR/CPCSEA/IAEC/2022/P.Col/R6) complying with the regulations of CPCSEA or Committee for the Purpose of Control and Supervision of Experiments on Animals. Moreover, the experimentation methods followed the guidelines for the care of laboratory animals as outlined by the National Research Council US Committee for Update of the Guide for the Care and Use of Laboratory Animals [38].

2.3. Induction of diabetes mellitus in animals

Injection of STZ (50 mg/kg, intraperitoneally) [9,39] was administered to fasted rodents to markedly increase the amount of blood glucose in experimental animals. The glucose level in blood was evaluated after seven days of STZ injection spectrophotometrically at 505 nm by using an enzymatic kit (Glucose estimation kit- 93DP100-74, ARKRAY Healthcare Pvt. Ltd., Surat, India) through GOD/POD (glucose oxidase/pyruvate oxidase) method [40,41]. Animals' blood glucose levels≥ 200 mg/dL are recognized to be diabetic. Six weeks post STZ injection, the animals participated in the research.

2.4. Experimental procedure

The experimental procedure is shown in schematic Fig-1 (A) and (B). The animals were kept under the standard condition and categorized into nine groups comprising six animals in each namely Sham, I/R, I/R + Chry, IPC, DM + IPC, DM + Chry + IPC, DM + Chry + IPC + LY, DM + Chry + IPC + L-NAME and DM + Chry + IPC + LY + L-NAME in the present experimental protocol. Chrysin was given via oral route at a dose of 60 mg/kg [42] for a week before the isolation of the heart to all the animals except Sham, I/R, IPC, and DM + IPC group rodents. After 1 h of the last dose of Chrysin, animals were dissected and the heart was swiftly excised for mounting on Langendorff's apparatus. Moreover, LY294002 (10 μM) [43] and L-NAME (100 μM/L) [44] was perfused for 5 min of reperfusion phase of each IPC cycle to DM + Chry + IPC + LY, DM + Chry + IPC + L-NAME, and DM + Chry + IPC + LY + L-NAME group animals respectively to evaluate the role of PI3K and NO. In each group, experiment-isolated rat hearts were stabilized for 10 min using Langendorff's equipment. The heart of animals in the control group was subjected to perfusion for a duration of 190 min.

Following the stabilization phase, the hearts in the I/R group experienced a 30-min period of ischemic challenge, succeeded by 120 min of reperfusion using K–H buffer solution which was freshly prepared. The heart exposed to IPC experienced a repetitive pattern of 5-min intervals of ischemia and reperfusion, which was then followed by a continuous 30-min period of ischemia and a subsequent 120-min reperfusion phase. This same protocol was administered to all hearts in the other animals within each group.

2.5. Isolated heart preparation

Heparin (250 U/kg; i.p.) was injected into the experimental animals to prevent clotting in the heart after that we swiftly excised the heart, then promptly placed it onto Langendorff's apparatus. This excised heart was put in an ice-cold K–H buffer solution with 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20 mM sodium acetate, 1.7 mM $CaCl_2$, as well as 10 mM glucose, with a pH of 7.4. It was then exposed to an environment with O_2 (95 %) as well as CO_2 (5 %), at 37 °C, and enclosed within a dual-walled jacket with water in circulation (37 °C). We collected coronary effluents to measure LDH and CK-MB activity, as well as nitrite concentration in the coronary flow [11].

2.6. Cellular injury estimation

The extent of myocardial damage was assessed by analyzing the levels of LDH as well as CK-MB in coronary flow samples obtained at different time intervals. The LDH as well as CK-MB levels in the perfusate were estimated through spectrophotometry after the experiment using commercial LDH (LDH-74LS100-25, Span Diagnostics Ltd., Gujarat, India) and CK-MB (CK-MB-1102070210, Coral Clinical Systems, Goa, India) detection kits [11].

2.7. Estimation of myocardial infarct area

The de-mounted heart was rinsed with phosphate buffer saline, frozen, stored (−20 °C) for half an hour, and then sliced (1 mm thick) perpendicularly from the apex to the base. According to earlier publications, these slices underwent incubation in a 1 % TTC with (pH 7.4 as well as temperature- 37 °C) for 10–15 min before being preserved in a 10 % formaldehyde solution [9,11]. The infarcted area remained unstained, but the surrounding normal myocardium showed a brick-red colour. The percentage infarct area (IA) was calculated as (%IA = IA/total area of slice × 100).

2.8. Estimation of nitrite

The level of nitrite was estimated through the standard protocol in coronary flow collected at different time points [45,46]. The level of nitrite was expressed in μM.

3. Assessment of oxidative stress

3.1. Estimation of LPO activity

The malonaldehyde content (MDA) was used to estimate lipid peroxidation. The content of MDA was assessed using colourimetry at a wavelength of 532 nm in the isolated heart and the measurements were given as μM of MDA/mg protein [47].

3.2. SOD activity estimation

Superoxide dismutase (SOD) activity is dependent on the formazan activity of NADH-phenazine methosulfate-nitro blue tetrazolium (NBT), which was quantified using spectrophotometry at a wavelength of 560 nm. One unit of the enzyme was defined as a fifty per cent reduction in NBT per minute per mg protein within the given assay parameters [48].

3.3. Catalase (CAT) activity estimation

Hydrogen peroxide was decomposed by catalase enzyme present in the isolated heart tissue, and it was detected by using a spectrophotometer at 240 nm. Hence, the findings were shown as CAT activity units per minute per mg protein [49].

3.4. Analysis of the data

A two-way analysis of variance (ANOVA) was performed, and then Bonferroni's post hoc test was performed. The statistical analysis of coronary flow, nitrite enzyme levels, LDH and CK activities in the animals' coronary effluent was conducted using the Bonferroni's test. One-way ANOVA was utilised for any subsequent statistical data analysis, and the Student Newman-Keuls post hoc test was then employed to determine group significance. A significance level of $P < 0.05$ was noted.

4. Result

4.1. STZ injection enhances the serum glucose level

Fig-2 illustrates the impact of streptozotocin on serum glucose levels. Following STZ administration, there is a substantial elevation in glucose levels of serum upon comparison to the control.

4.2. Chrysin restores the effect of IPC in I/R injury-stimulated decreased coronary flow in diabetic rats' heart

Chrysin's actions on IPC over I/R injury-induced alteration in coronary flow in the hearts of diabetic-challenged rats is displayed in Fig-3. The statistical analysis indicated the presence of noteworthy distinctions among the groups [$F_{8,\ 45} = 43.99$, $P < 0.05$], time [$F_{3,\ 135} = 418.2$, $P < 0.05$], along with a notable relation of experimental group and time [$F_{24,\ 135} = 13.43$, $P < 0.05$]. Further, Bonferroni's test revealed no notable variations in coronary blood flow during the basal duration between the rat groups. Nonetheless, IPC failed to remarkably attenuate the diabetes-induced decrease in coronary flow rate. Moreover, chrysin significantly reversed the effect of IPC in terms of an elevation in coronary flow rate in diabetic-challenged rat hearts. However, LY294002 and L-NAME reversed the effect of chrysin in relation to the decline in coronary flow during the experiment in diabetic rat hearts. Over the course of the 120-min experiment plan, this effect persisted.

4.3. Chrysin restores the effect of IPC in I/R injury-stimulated rise in LDH level in diabetic rat heart

Fig-4 depicts the effect of chrysin on IPC against I/R injury-triggered alteration in the level of LDH in diabetic-challenged rat hearts. The statistical analysis demonstrated a notable distinction between the groups [$F_{8,\ 45} = 31.32$, $P < 0.05$], time [$F_{2,\ 90} = 350.5$, $P < 0.05$], and a noticeable connection in group and time [$F_{16,\ 90} = 16.02$, $P < 0.05$]. Furthermore, the post hoc evaluation displayed no remarkable variation in the level of LDH during basal time among the groups. However, IPC failed to significantly attenuate the diabetes-induced increase in LDH levels. Moreover, chrysin significantly restored the effect of IPC in relation to a reduction in LDH levels of diabetic-challenged rat hearts. However, LY294002 and L-NAME reverse the effect of chrysin in terms of a rise in LDH level during the experiment in diabetic-challenged rat hearts.

4.4. Chrysin restores the effect of IPC in I/R injury-triggered rise in CK-MB level in diabetic rat heart

The effect of chrysin on IPC against I/R injury-stimulated alteration in CK-MB level of diabetic-challenged animal hearts is presented in Fig-5. The statistical analysis indicated significant variations among the groups [$F_{8,\ 45} = 17.84$, $P < 0.05$], time [$F_{1,\ 45} = 816.0$, $P < 0.05$], and a significant connection between the animal groups as well as time [$F_{8,\ 45} = 31.51$, $P < 0.05$]. In addition, Bonfferoni's test revealed that there was no significant variation in CK-MB level during the baseline time between the groups. However, IPC failed to significantly reduce the diabetes-caused elevation in CK-MB levels. Moreover, chrysin dramatically reversed

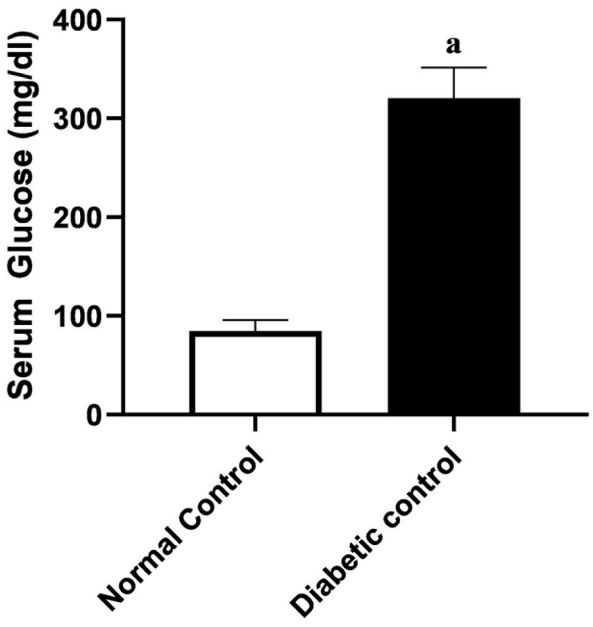

Fig-2. Effect of streptozotocin administration on the serum glucose level. All values are mean ± standard error mean (SEM; n = 6). [a]p<0.05 in comparison to normal control.

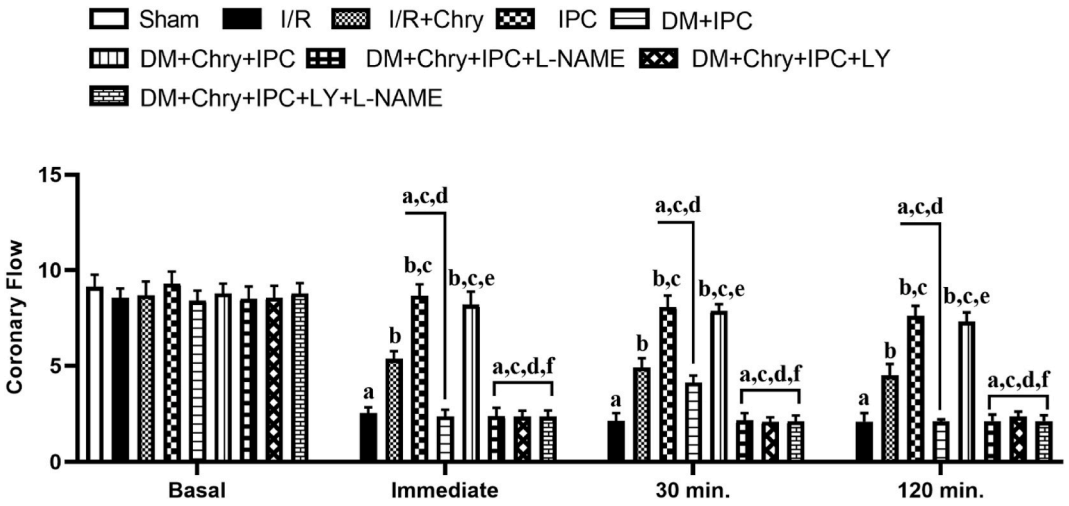

Fig-3. Effect of chrysin on IPC against I/R injury-triggered alteration in coronary flow of diabetic-challenged rat heart at different time points. All values are mean ± SEM (n = 6). [a]p<0.05 in comparison to Sham, [b]p < 0.05 compared to I/R, [c]p < 0.05 compared to I/R + Chry, [d]p < 0.05 in comparison to IPC, [e]p < 0.05 compared to DM + IPC, [f]p < 0.05 in comparison to DM + Chry + IPC (Two-way ANOVA subsequently followed by Bonferroni post-hoc analysis).

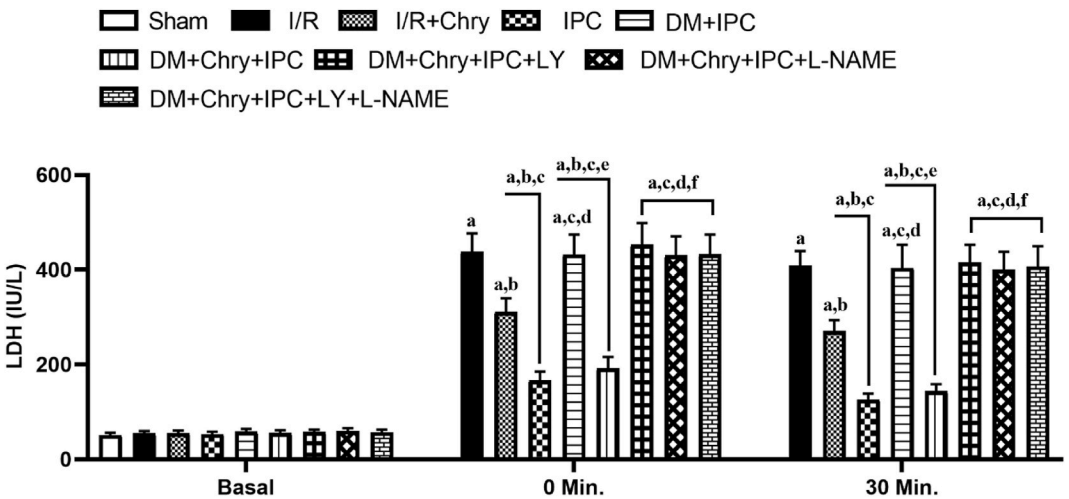

Fig-4. Effect of chrysin on IPC against I/R injury-triggered alteration in LDH level of diabetic-challenged rat heart at different time points. All values are mean ± SEM (n = 6). [a]p<0.05 in comparison to Sham, [b]p < 0.05 in comparison to I/R, [c]p < 0.05 in comparison to I/R + Chry, [d]p < 0.05 in comparison to IPC, [e]p < 0.05 in comparison to DM + IPC, [f]p < 0.05 in comparison to DM + Chry + IPC (Two-way ANOVA subsequently followed by Bonferroni post-hoc analysis).

IPC's impact in relation to suppression in CK-MB level of diabetic-challenged rat hearts. However, LY294002 and L-NAME reverse the effect of chrysin in terms of an increase in CK-MB level during the experiment in diabetic-challenged rat hearts.

4.5. Chrysin restores the impact of IPC in I/R injury-stimulated reduction in nitrite levels in diabetic rat heart

The effect of chrysin on IPC against I/R injury-triggered alteration in nitrite level of diabetic-challenged animal hearts is presented in Fig-6. Statistical data showed that significant variations exist among the experimental groups [$F_{8, 45} = 79.46$, $P < 0.05$], time [$F_{3, 135} = 403.1$, $P < 0.05$], as well as a notable relation among the experimental groups and duration (time) [$F_{24, 135} = 12.10$, $P < 0.05$]. Moreover, the post hoc evaluation revealed that no significant change was observed in the level of nitrite during basal time among the animal groups. However, IPC was not able to significantly decrease the diabetes-induced decrease in nitrite levels. Moreover, chrysin significantly neutralised IPC's effect in relation to an increase in nitrite levels of diabetic-challenged rat hearts. However, LY294002 and L-NAME reverse the effect of chrysin in terms of a decrease in nitrite level during the experiment in diabetic-challenged rat hearts.

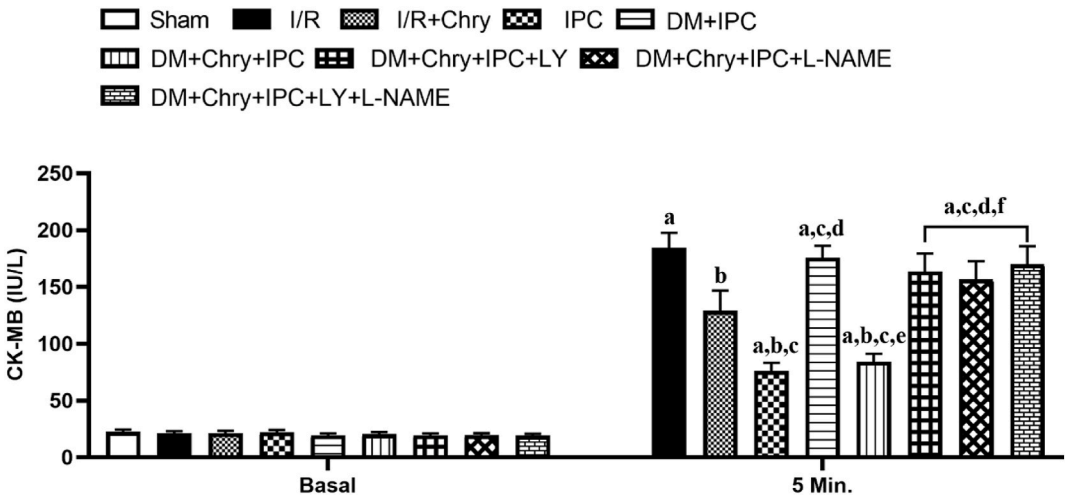

Fig-5. Effect of chrysin on IPC against I/R injury-stimulated alteration in CK-MB level of diabetic-challenged rat heart at different time points. All values are mean ± SEM (n = 6). [a]p<0.05 in comparison to Sham, [b]p < 0.05 in comparison to I/R, [c]p < 0.05 in comparison to I/R + Chry, [d]p < 0.05 in comparison to IPC, [e]p < 0.05 in comparison to DM + IPC, [f]p < 0.05 in comparison to DM + Chry + IPC (Two-way ANOVA subsequently followed by Bonferroni post-hoc analysis).

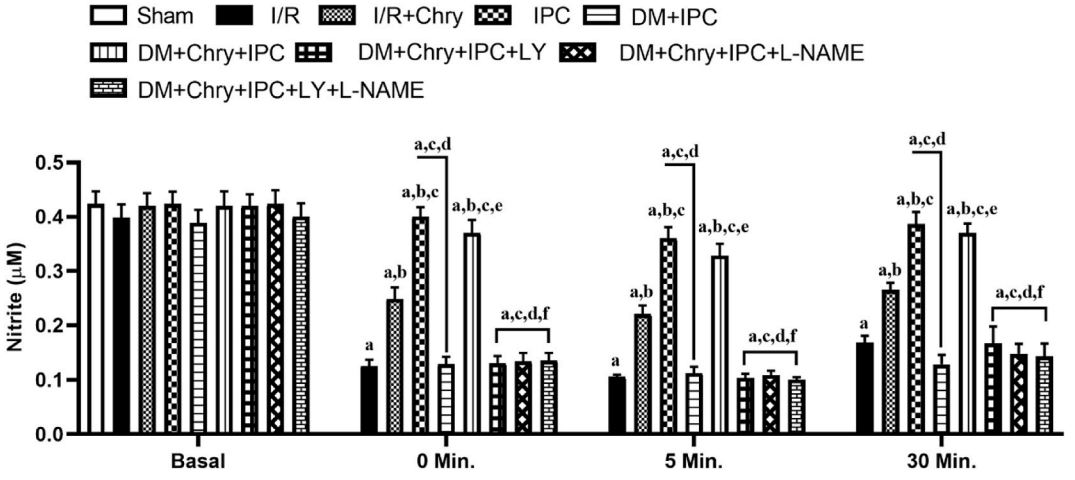

Fig-6. Effect of chrysin on IPC against I/R injury-stimulated variation in nitrite level of diabetic-challenged rat heart at different time points. All values are mean ± SEM (n = 6). [a]p<0.05 in comparison to Sham, [b]p < 0.05 in comparison to I/R, [c]p < 0.05 in comparison to I/R + Chry, [d]p < 0.05 in comparison to IPC, [e]p < 0.05 in comparison to DM + IPC, [f]p < 0.05 in comparison to DM + Chry + IPC (Two-way ANOVA subsequently followed by Bonferroni post-hoc analysis).

4.6. Chrysin restores the effect of IPC in I/R injury-induced rise in the size of infarct in the heart of diabetic rats

Chrysin's impact on IPC in I/R injury-induced alteration in infarct size in diabetic-challenged rat hearts is presented in Fig-7. Statistical evaluation displayed notable alterations among the animal groups [$F_{8,\ 45} = 31.16$, $P < 0.05$]. Further, the post hoc assessment displayed that IPC markedly reduced the I/R-triggered rise in the size of infarct. However, IPC was not able to significantly decrease the diabetes-induced increase in infarct size. Moreover, pre-treatment of chrysin reversed IPC's impact in relation to a suppression in the size of the infarct during I/R injury in diabetic-challenged rat hearts. However, LY294002 and L-NAME reverse the effect of chrysin in terms of the increase in infarct size during the experiment in diabetic-challenged rat hearts.

4.7. Chrysin restores the impact of IPC in I/R injury-triggered alteration in LPO, SOD, and CAT in diabetic rat heart

The effect of chrysin on IPC against I/R injury-induced alteration in the (A) LPO level and (B) SOD and (C) CAT activities in diabetic-challenged rat hearts is represented in Fig-8. Statistical evaluation showed a notable alteration in the LPO level along with SOD and CAT activities ([$F_{8,\ 45} = 30.88$, $P < 0.05$], [$F_{8,\ 45} = 27.53$, $P < 0.05$] and [$F_{8,\ 45} = 21.18$, $P < 0.05$], respectively). Moreover,

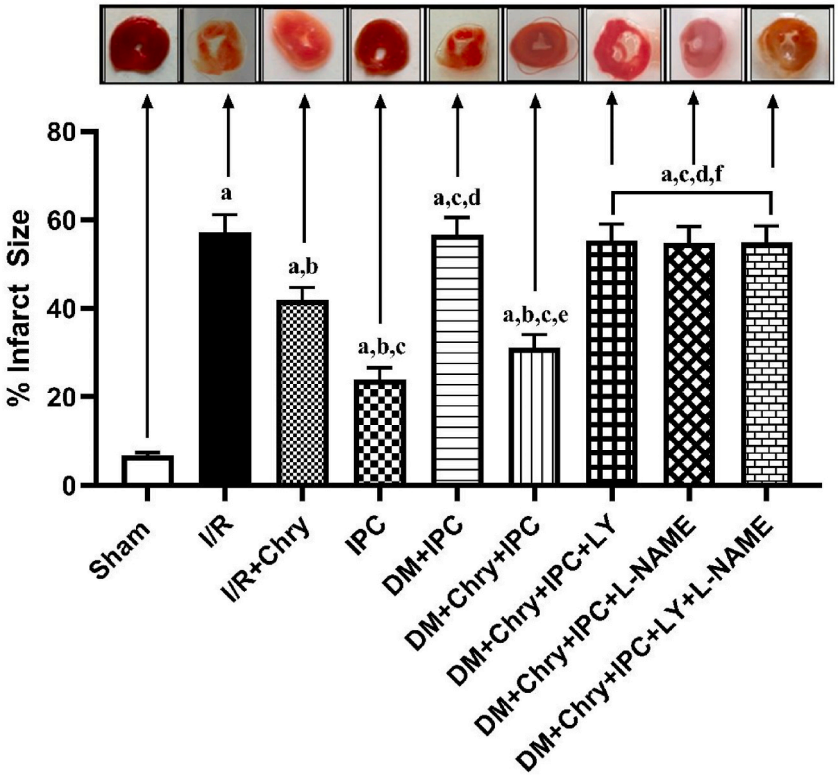

Fig-7. Effect of chrysin on IPC against I/R injury-triggered changes in infarct size in diabetic-challenged rat heart. All values are mean ± SEM (n = 6). [a]p<0.05 compared to Sham, [b]p < 0.05 compared to I/R, [c]p < 0.05 compared to I/R + Chry, [d]p < 0.05 compared to IPC, [e]p < 0.05 compared to DM + IPC, [f]p < 0.05 compared to DM + Chry + IPC (One-way ANOVA subsequently followed by Student Newman–Keuls Post hoc analysis).

Bonfferoni's test displayed that IPC remarkably reduced I/R-stimulated rise and fall in the LPO level along with the activities of SOD and CAT, respectively. However, IPC was not able to significantly mitigate the diabetes-induced elevation in infarct size. Moreover, pre-treatment of chrysin neutralised IPC's action in relation to suppression and elevation in the level of LPO as well as the SOD and CAT activities, respectively in I/R injury in diabetic-challenged rat hearts. However, LY294002 and L-NAME reverse the effect of chrysin during the experiment in diabetic-challenged rat hearts.

5. Discussion

The present work demonstrates the stimulation of the PI3K-Akt-eNOS signalling mechanism by which chrysin renewed the effect of IPC against I/R-triggered elevation in oxidative stress, LDH levels, infarct size as well as CK-MB levels, and decreased nitrite level in diabetic-challenged rat heart. These observations were confirmed through the perfusion of LY294002 and L-NAME (PI3K and eNOS inhibitor) which notably attenuated the chrysin-stimulated restoration of IPC via PI3K-Akt-eNOS pathway in the isolated diabetic animal heart. Therefore, for the first time, it can be inferred that chrysin has the potential to reinstate IPC's effectiveness to prevent I/R injury via PI3K-Akt-eNOS signalling in diabetic rats.

Ischemia-reperfusion is an experimental model that is widely recognized for investigating the mechanism of various drugs, given the same biological-chemical changes observed in patients who were suffering from myocardial infarction [50–52]. The present study demonstrates that I/R injury caused a decline in the coronary flow. Moreover, augmented myocardial infarct size, levels of LDH as well as CK-MB, and suppressed level of nitrite which was collected in the coronary flow at various intervals have shown similar results as per the previous studies of our laboratory [11]. However, IPC a healthy heart phenomenon protected the myocardium against I/R injury-induced alterations. Nonetheless, in this study, the effect of the healthy heart phenomenon significantly attenuated in the diabetic condition which is similar to our previously published reports [53]. The originality of the current work is that pre-treatment of chrysin restored the beneficial actions of IPC in mitigating I/R-induced alterations which may be due to an increase in nitric oxide level [54]. Other probable explanations include the triggering of the PI3K/Akt route, which is a very promising pathway to show cardioprotection, which is already reported by our laboratory [12,34]. These above-mentioned observations were confirmed through the administration of LY294002 and L-NAME with chrysin, which showed that the protective effect of IPC was lost in relevant groups. According to the above-mentioned observations, chrysin may be a preferable supplement choice for myocardial injury management in diabetic animals.

The generation of reactive oxygen species (ROS) is the major culprit for the progression and development of myocardial damage

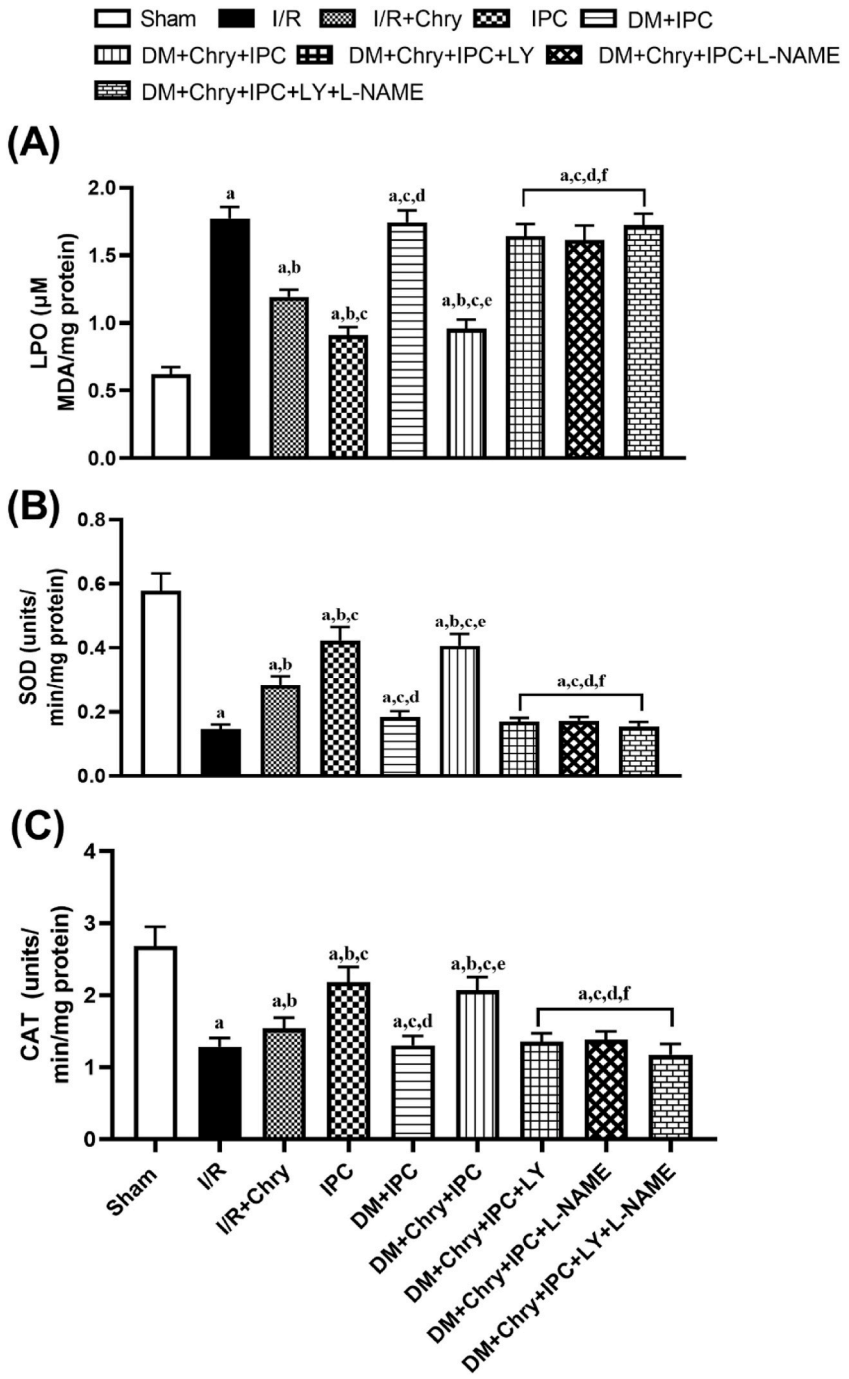

Fig-8. Effect of chrysin on IPC against I/R injury-triggered variation in the level of (A) LPO and activities of (B) SOD and (C) CAT of diabetic-challenged rat heart. All values are mean ± SEM (n = 6). [a]p<0.05 in comparison to Sham, [b]p < 0.05 compared to I/R, [c]p < 0.05 in comparison to I/R + Chry, [d]p < 0.05 compared in comparison to IPC, [e]p < 0.05 in comparison to DM + IPC, [f]p < 0.05 in comparison to DM + Chry + IPC (One-way ANOVA subsequently followed by Student Newman–Keuls Post hoc analysis).

during diabetes [55]. In our study, free radical production was observed during the I/R injury, free radicals consume the antioxidants such as catalase leading to a disproportion between the oxidants as well as antioxidants within the myocyte, ultimately resulting in myocyte death [50,51]. In our study, pre-treatment of chrysin restored the effect of IPC in I/R injury in relation to an increase in activities of SOD, CAT, along with a reduction in LPO level in diabetic-challenged rat hearts. However, LY294002 and L-NAME reverse the effect of chrysin during the experiment in diabetic-challenged rat hearts. Interestingly, this antioxidant effect of chrysin was attributed to the existence of the hydroxyl group [56]. Additionally, reduction in oxidative strain through the stimulation of PI3K-Akt

cascade is an important mechanism of chrysin [57]. These findings infer that chrysin restored the cardioprotective activity of IPC perhaps through its antioxidant status via PI3K-Akt-eNOS pathway against I/R injury in diabetic-challenged animals.

6. Conclusion

In conclusion, the rejuvenating impact of IPC to manage I/R injury in diabetic experimental rat hearts was exhibited by chrysin, and this was achieved through the PI3K-Akt-eNOS cascade. Further, through the PI3K-Akt-eNOS pathway, chrysin rescued the impact of IPC in terms of correcting antioxidant status. Additionally, chrysin improves the coronary flow, and nitrite level, and suppresses the levels of LDH, CK-MB, and the size of the infarct in the hearts of diabetic rats. However, LY294002 and L-NAME reversed the protective effect of chrysin in terms of the above-mentioned biochemical observations in diabetic rats' hearts. Hence, these findings suggest that chrysin may serve as a promising substitute supplement to restore IPC's protective effect against I/R injury in diabetic animals.

Compliance with ethical standards

All experimental methods employed were executed in compliance with the Institutional Animal Ethics Committee's approval (GLAIPR/CPCSEA/IAEC/2022/P.Col/R6), strictly adhering to the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for research studies.

Data availability statement

Data has been included in the article.

CRediT authorship contribution statement

Geetanjali Singh: Conceptualization. **Vibhav Varshney:** Methodology. **Ahsas Goyal:** Conceptualization, Formal analysis, Writing – review & editing. **Nemat Ali:** Methodology, Validation. **Muzaffar Iqbal:** Methodology, Visualization, Writing – review & editing. **Ishnoor Kaur:** Methodology, Writing – original draft. **Celia Vargas-De-La-Cruz:** Methodology, Writing – review & editing. **Tapan Behl:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M.A. Khan, M.J. Hashim, H. Mustafa, M.Y. Baniyas, S.K.B.M. Al Suwaidi, R. Al Katheeri, et al., Global epidemiology of ischemic heart disease: results from the global burden of disease study, Cureus 12 (7) (2020) e9349, https://doi.org/10.7759/cureus.9349.
[2] S. Johansson, A. Rosengren, K. Young, E. Jennings, Mortality and morbidity trends after the first year in survivors of acute myocardial infarction: a systematic review, BMC Cardiovasc. Disord. 17 (1) (2017) 53, https://doi.org/10.1186/s12872-017-0482-9.
[3] R.K. Srivastav, T.M. Ansari, M. Prasad, V.K. Vishwakarma, P.K. Upadhyay, F. Ahsan, et al., An inquest into regulatory mechanism of caveolin by ischemic preconditioning against orchidectomy-challenged rat heart, Mol. Cell. Biochem. 476 (7) (2021) 2587–2601, https://doi.org/10.1007/s11010-021-04109-1.
[4] V. Agrawal, J.K. Gupta, S.S. Qureshi, V.K. Vishwakarma, Role of cardiac renin angiotensin system in ischemia reperfusion injury and preconditioning of heart, Indian Heart J. 68 (6) (2016) 856–861, https://doi.org/10.1016/j.ihj.2016.06.010.
[5] L.H. Snoeckx, G.J. van der Vusse, W.A. Coumans, P.H. Willemsen, R.S. Reneman, Differences in ischaemia tolerance between hypertrophied hearts of adult and aged spontaneously hypertensive rats, Cardiovasc. Res. 27 (5) (1993) 874–881, https://doi.org/10.1093/cvr/27.5.874.
[6] A.S. Go, D. Mozaffarian, V.L. Roger, E.J. Benjamin, J.D. Berry, W.B. Borden, et al., American heart association statistics committee and stroke statistics subcommittee. Heart disease and stroke statistics, update: a report from the American heart association, Circulation 127 (1) (2013) e6–e245, https://doi.org/10.1161/CIR.0b013e31828124ad.
[7] M. Liu, P. Zhang, M. Chen, W. Zhang, L. Yu, X.C. Yang, et al., Aging might increase myocardial ischemia/reperfusion-induced apoptosis in humans and rats, Age (Dordr). 34 (3) (2012) 621–632, https://doi.org/10.1007/s11357-011-9259-8.
[8] J. Marczak, R. Nowicki, J. Kulbacka, J. Saczko, Is remote ischaemic preconditioning of benefit to patients undergoing cardiac surgery? Interact. Cardiovasc. Thorac. Surg. 14 (5) (2012) 634–639, https://doi.org/10.1093/icvts/ivr123.
[9] H.N. Yadav, M. Singh, P.L. Sharma, Involvement of GSK-3β in attenuation of the cardioprotective effect of ischemic preconditioning in diabetic rat heart, Mol. Cell. Biochem. 343 (1–2) (2010) 75–81, https://doi.org/10.1007/s11010-010-0500-z.
[10] P. Cowled, R. Fitridge, Pathophysiology of reperfusion injury, in: R. Fitridge, M. Thompson (Eds.), Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists, University of Adelaide Press, Adelaide (AU), 2011.
[11] P. Pachauri, D. Garabadu, A. Goyal, P.K. Upadhyay, Angiotensin (1-7) facilitates cardioprotection of ischemic preconditioning on ischemia-reperfusion-challenged rat heart, Mol. Cell. Biochem. 430 (1–2) (2017) 99–113, https://doi.org/10.1007/s11010-017-2958-4.
[12] V. Varshney, A. Goyal, J. Gupta, H.N. Yadav, Role of erythropoietin in ischemic postconditioning induced cardioprotection in hyperlipidemic rat heart, J. Indian Coll. Cardiol. 7 (2) (2017) 72–77.

[13] A. Goyal, N. Agrawal, Ischemic preconditioning: interruption of various disorders, J. Saudi Heart Assoc. 29 (2) (2017) 116–127, https://doi.org/10.1016/j.jsha.2016.09.002.

[14] H.N. Yadav, M. Singh, P.L. Sharma, Modulation of the cardioprotective effect of ischemic preconditioning in hyperlipidaemic rat heart, Eur. J. Pharmacol. 643 (1) (2010) 78–83, https://doi.org/10.1016/j.ejphar.2010.06.015.

[15] A. Lejay, F. Fang, R. John, J.A. Van, M. Barr, F. Thaveau, et al., Ischemia reperfusion injury, ischemic conditioning and diabetes mellitus, J. Mol. Cell. Cardiol. 91 (2016) 11–22, https://doi.org/10.1016/j.yjmcc.2015.12.020.

[16] G. Danaei, M.M. Finucane, Y. Lu, G.M. Singh, M.J. Cowan, C.J. Paciorek, et al., National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants, Lancet 378 (9785) (2011) 31–40, https://doi.org/10.1016/S0140-6736(11)60679-X.

[17] G. Danaei, C.M. Lawes, S. Vander Hoorn, C.J. Murray, M. Ezzati, Global and regional mortality from ischaemic heart disease and stroke attributable to higher-than-optimum blood glucose concentration: comparative risk assessment, Lancet 368 (9548) (2006) 1651–1659, https://doi.org/10.1016/S0140-6736(06)69700-6.

[18] G.M. Singh, G. Danaei, F. Farzadfar, G.A. Stevens, M. Woodward, D. Wormser, et al., The age-specific quantitative effects of metabolic risk factors on cardiovascular diseases and diabetes: a pooled analysis, PLoS One 8 (7) (2013), e65174, https://doi.org/10.1371/journal.pone.0065174.

[19] R. Rohilla, A. Goyal, V. Varshney, B.C. Semwal, H.N. Yadav, Role of heme oxygenase- 1(HO-1) and endothelin-1 (ET-1) in modulation of cardioprotective effect of ischemic postconditioning in diabetic rat heart, Indian J. of Pharmaceutical Education and Research 54 (3) (2020) 690–697, https://doi.org/10.5530/ijper.54.3.119.

[20] H. Yadav, V. Varshney, N. Singh, P.L. Sharm, Quercetin: a phytoestrogen attenuate GSK-3β inhibitors induced delayed cardioprotection in diabetic rat heart, Pharmacologia 6 (2015) 293–299, https://doi.org/10.17311/pharmacologia.2015.293.299.

[21] H. Hotta, T. Miura, T. Miki, N. Togashi, T. Maeda, S.J. Kim, Angiotensin II type 1 receptor-mediated upregulation of calcineurin activity underlies impairment of cardioprotective signaling in diabetic hearts, Circ. Res. 106 (1) (2010) 129–132, https://doi.org/10.1161/CIRCRESAHA.109.205385.

[22] Y. Liu, J.D. Thornton, M.V. Cohen, J.M. Downey, S.W. Schaffer, Streptozotocin-induced non-insulin-dependent diabetes protects the heart from infarction, Circulation 88 (3) (1993) 1273–1278, https://doi.org/10.1161/01.cir.88.3.1273.

[23] T. Tatsumi, S. Matoba, M. Kobara, N. Keira, A. Kawahara, K. Tsuruyama, Energy metabolism after ischemic preconditioning in streptozotocin-induced diabetic rat hearts, J. Am. Coll. Cardiol. 31 (3) (1998) 707–715, https://doi.org/10.1016/s0735-1097(97)00556-1.

[24] M. Thirunavukkarasu, S.V. Penumathsa, S. Koneru, B. Juhasz, L. Zhan, H. Otani, Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: role of nitric oxide, thioredoxin, and heme oxygenase, Free Radic. Biol. Med. 43 (5) (2007) 720–729, https://doi.org/10.1016/j.freeradbiomed.2007.05.004.

[25] B. Walkowski, M. Kleibert, M. Majka, M. Wojciechowska, Insight into the role of the PI3K/akt pathway in ischemic injury and post-infarct left ventricular remodeling in normal and diabetic heart, Cells 11 (9) (2022) 1553, https://doi.org/10.3390/cells11091553.

[26] F.L. Zhang, S.L. Chu, W.W. Wang, L.L. Chen, [Downregulated PI3K-Akt-eNOS expression is related to increased atrial fibrillation inducibility in diabetic rats], Zhonghua Xinxueguanbing Zazhi 46 (5) (2018) 376–381, https://doi.org/10.3760/cma.j.issn.0253-3758.2018.05.010.

[27] A. Garg, S. Chaturvedi, A comprehensive review on chrysin: emphasis on molecular targets, pharmacological actions and bio-pharmaceutical aspects, Curr. Drug Targets 23 (4) (2022) 420–436, https://doi.org/10.2174/1389450122666210824141044.

[28] A. Gil-Ramírez, C. Pavo-Caballero, E. Baeza, N. Baenas, C. Garcia-Viguera, F.R. Marín, et al., Mushrooms do not contain flavonoids, J. Funct.Foods 25 (2016) 1–13, https://doi.org/10.1016/j.jff.2016.05.005.

[29] I.C. Ferreira, L. Barros, R.M. Abreu, Antioxidants in wild mushrooms, Curr. Med. Chem. 16 (12) (2009) 1543–1560, https://doi.org/10.2174/092986709787909587.

[30] R. Mani, V. Natesan, Chrysin: sources, beneficial pharmacological activities, and molecular mechanism of action, Phytochemistry 145 (2018) 187–196, https://doi.org/10.1016/j.phytochem.2017.09.016.

[31] S.S. Tian, F.S. Jiang, K. Zhang, X.X. Zhu, B. Jin, J.J. Lu, et al., Flavonoids from the leaves of Caryacathayensis Sarg. inhibit vascular endothelial growth factor-induced angiogenesis, Fitoterapia 92 (2014) 34–40, https://doi.org/10.1016/j.fitote.2013.09.016.

[32] R. Anandhi, P.A. Thomas, P. Geraldine, Evaluation of the anti-atherogenic potential of chrysin in Wistar rats, Mol. Cell. Biochem. 385 (1–2) (2014) 103–113, https://doi.org/10.1007/s11010-013-1819-z.

[33] S. Samarghandian, M. Azimi-Nezhad, F. Samini, T. Farkhondeh, Chrysin treatment improves diabetes and its complications in liver, brain, and pancreas in streptozotocin-induced diabetic rats, Can. J. Physiol. Pharmacol. 94 (4) (2016) 388–393, https://doi.org/10.1139/cjpp-2014-0412.

[34] M. Talebi, M. Talebi, T. Farkhondeh, J. Simal-Gandara, D.M. Kopustinskiene, J. Bernatoniene, et al., Promising protective effects of chrysin in cardiometabolic diseases, Curr. Drug Targets 23 (5) (2022) 458–470, https://doi.org/10.2174/1389450122666211005113234.

[35] T.F. Li, J. Ma, X.W. Han, Y.X. Jia, H.F. Yuan, S.F. Shui, et al., Chrysin ameliorates cerebral ischemia/reperfusion (I/R) injury in rats by regulating the PI3K/Akt/mTOR pathway, Neurochem. Int. 129 (2019), 104496, https://doi.org/10.1016/j.neuint.2019.104496.

[36] S. Yuvaraj, T. Ramprasath, B. Saravanan, V. Vasudevan, S. Sasikumar, G.S. Selvam, et al., Chrysin attenuates high-fat-diet-induced myocardial oxidative stress via upregulating eNOS and Nrf2 target genes in rats, Mol. Cell. Biochem. 476 (7) (2021) 2719–2727, https://doi.org/10.1007/s11010-021-04105-5.

[37] T. Farkhondeh, S. Samarghandian, F. Bafandeh, The cardiovascular protective effects of chrysin: a narrative review on experimental researches. Cardiovasc hematol agents, Med. Chem. 17 (1) (2019) 17–27, https://doi.org/10.2174/1871525717666190114145137.

[38] National Research Council (Us) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011) Guide for the Care and Use of Laboratory Animals, 8th edn. National Academies Press (US), Washington, DC..

[39] G. Ozansoy, F.B. Akin, Effects of gemfibrozil treatment on vascular reactivity of streptozotocin-diabetic rat aorta, J. Pharm. Pharmacol. 56 (2) (2004) 241–246, https://doi.org/10.1211/0022357022737.

[40] P. Trinder, Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen, J. Clin. Pathol. 22 (2) (1969) 158–161, https://doi.org/10.1136/jcp.22.2.158.

[41] J.A. Lott, K. Turner, Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine, Clin. Chem. 21 (12) (1975) 1754–1760.

[42] N. Rani, S. Bharti, J. Bhatia, T.C. Nag, R. Ray, D.S. Arya, et al., Chrysin, a PPAR-γ agonist improves myocardial injury in diabetic rats through inhibiting AGE-RAGE mediated oxidative stress and inflammation, Chem. Biol. Interact. 250 (2016) 59–67, https://doi.org/10.1016/j.cbi.2016.03.015.

[43] A. Tsang, D.J. Hausenloy, M.M. Mocanu, R.D. Carr, D.M. Yellon, Preconditioning the diabetic heart: the importance of Akt phosphorylation, diabetes 54 (8) (2005) 2360–2364, https://doi.org/10.2337/diabetes.54.8.2360.

[44] A. Goyal, B.C. Semwal, H.N. Yadav, Abrogated cardioprotective effect of ischemic preconditioning in ovariectomized rat heart, Hum. Exp. Toxicol. 35 (6) (2016) 644–653, https://doi.org/10.1177/0960327115597980.

[45] M.A. Marletta, P.S. Yoon, R. Iyengar, C.D. Leaf, J.S. Wishnok, Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate, Biochemistry 27 (24) (1988) 8706–8711, https://doi.org/10.1021/bi00424a003.

[46] V. Parikh, M. Singh, Possible role of nitric oxide and mast cells in endotoxin-induced cardioprotection, Pharmacol. Res. 43 (1) (2001) 39–45, https://doi.org/10.1006/phrs.2000.0750.

[47] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (2) (1979) 351–358, https://doi.org/10.1016/0003-2697(79)90738-3.

[48] P. Kakkar, B. Das, P.N. Viswanathan, A modified spectrophotometric assay of superoxide dismutase, Indian J. Biochem. Biophys. 21 (2) (1984) 130–132.

[49] R.F. Jr Beers, I.W. Sizer, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, J. Biol. Chem. 195 (1) (1952) 133–140.

[50] S. Cadenas, ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection, Free Radic. Biol. Med. 117 (2018) 76–89, https://doi.org/10.1016/j.freeradbiomed.2018.01.024.

[51] D.J. Hausenloy, D.M. Yellon, Myocardial ischemia-reperfusion injury: a neglected therapeutic target, J. Clin. Invest. 123 (1) (2013) 92–100, https://doi.org/10.1172/JCI62874.

[52] V.K. Vishwakarma, P.K. Upadhyay, J.K. Gupta, H.N. Yadav, Pathophysiologic role of ischemia reperfusion injury: a review, Journal of Indian College of Cardiology 7 (3) (2017) 97–104, https://doi.org/10.1016/j.jicc.2017.06.017.
[53] K. Charan, A. Goyal, J.K. Gupta, H.N. Yadav, Role of atrial natriuretic peptide in ischemic preconditioning-induced cardioprotection in the diabetic rat heart, J. Surg. Res. 201 (2) (2016) 272–278, https://doi.org/10.1016/j.jss.2015.10.045.
[54] R. Veerappan, T. Malarvili, Chrysin pretreatment improves angiotensin system, cGMP concentration in L-NAME induced hypertensive rats, Indian J. Clin. Biochem. 34 (3) (2019) 288–295, https://doi.org/10.1007/s12291-018-0761-y.
[55] N. Rani, S. Bharti, J. Bhatia, T.C. Nag, R. Ray, D.S. Arya, et al., Chrysin, a PPAR-γ agonist improves myocardial injury in diabetic rats through inhibiting AGE-RAGE mediated oxidative stress and inflammation, Chem. Biol. Interact. 250 (2016) 59–67, https://doi.org/10.1016/j.cbi.2016.03.015.
[56] G.K. Harris, Y. Qian, S.S. Leonard, D.C. Sbarra, X. Shi, Luteolin and chrysin differentially inhibit cyclooxygenase-2 expression and scavenge reactive oxygen species but similarly inhibit prostaglandin-E2 formation in RAW 264.7 cells, J. Nutr. 136 (6) (2006) 1517–1521, https://doi.org/10.1093/jn/136.6.1517.
[57] Y. Li, X. Wang, Chrysin attenuates high glucose-induced BMSC dysfunction via the activation of the PI3K/AKT/Nrf2 signaling pathway, Drug Des. Devel. Ther. 16 (2022) 165–182, https://doi.org/10.2147/DDDT.S335024. . Fig-1.