Role of Irisin in Ischaemia - Reperfusion Injury in Isolated Heart of Albino Rats

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Abstract: **Background:** Irisin, a newly identified myokine, is critical in modulating body metabolism, thermogenesis and reducing oxidative stresses. Lower plasma level of irisin in patients with acute myocardial infarction (AMI) was reported. Nevertheless, the significance and functional role of irisin in the modulation of myocardial ischemia and reperfusion injury are not clear. **Objective:** This study was designed to explore possible effect of irisin on ischemia-reperfusion injury in isolated heart of adult male albino rats and to explain the possible involved mechanisms, in a trial to clarify irisin expected cardioprotective effect. **Design**: This study was carried out on thirty sex adult male albino rats which were divided equally (n=12) into 3 groups: Group I (ischemia-reperfusion I/R group); hearts were stabilized then subjected to (I/R) protocol, Group II (Irisin pre-conditioning group); Irisin was infused for 20 minutes before hearts were subjected to ischemia and Group III (Irisin post-conditioning group); Irisin was infused for 20 minutes at the beginning of 60 minutes of reperfusion. Cardiac performance indicators as left ventricular pressure (LVP), +max (LV dp/dt), -max (LVdP/dt), in addition to heart rate were recorded. Lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), superoxide dismutase (SOD) and C-reactive protein (CRP) were measured in the collected perfusate and cardiac Malondialdehyde (MDA) was measured. Finally, Nitro blue tetrazolium stain was used to detect the necrotic tissue percentage to the whole left ventricular mass. **Results**: In group III (post conditioning group), there was a significant increase of the studied cardiac parameters compared to group I (I/R). Irisin significantly increased LVP, +max (dp/dt), -max (dp/dt) and HR in comparison with I/R group. This was associated with a significant decrease in LDH and CK-MB levels, a significant increase in SOD level and a significant decrease in MDA and CRP levels. Moreover, Irisin caused a significant decrease in percentage of necrotic tissue to the whole left ventricular mass. Regarding group II, no significant changes were detected in all parameters when compared to group I. **Conclusion**: Irisin could protect against ischemia/ reperfusion injury in vitro through its antioxidant and anti-inflammatory properties, by limiting the infarction area, only if given as a post conditioning factor after I/R. Those results open the way to include Irisin among the strategies for management of cardiac infarction during reperfusion.

Keywords: Irisin; Myokines; MI; Heart; Cardioprotection; Ischemia/Reperfusion

I. INTRODUCTION

Ischemic heart disease is considered to be the leading cause of death worldwide and is predicted to be the major cause of deaths in the future [1]. Severe and prolonged ischemia ultimately results in myocardial damage and cellular necrosis [2]. Although rapid restoration of blood flow to the ischemic heart is the treatment of choice for reducing acute myocardial ischemic injury and limiting myocardial infarction (MI) size, it was proved that reperfusion per se may augment tissue injury which is known as reperfusion injury, for which there is still no effective therapy [2,3].

Moreover, it is possible to say that oxidative and inflammatory responses are extremely important in ischemia-reperfusion (I/R) injury phenomenon [3,4].At present, some drugs such as erythropoietin, adenosine, and hydrogen sulfide have shown good protective effect at the beginning of reperfusion of the heart, but these drugs have not been widely used clinically [1].

Exercise is the corner stone of a healthy lifestyle and has an important role in preventing cardiovascular diseases. Moreover, it is now established that skeletal muscle produces and secretes many hormones, which have been named "myokines", which subsequently exert autocrine, paracrine and / or endocrine effects [5,6].

Irisin is a recently discoverd exercise-induced myokine [5]. It is produced by the proteolytic processing of a transmembrane receptor (FNDC5), highly expressed in skeletal muscle, pericardium, heart, and brain, which produce its effects by binding to an unknown receptor [3, 5, 7, 8].

The beneficial effects of irisin on the metabolic syndromes produced not only though browning of white adipose tissue and increasing the energy expenditure, but also suppressing inflammation and oxidative stress were reported by many different studies [5, 9, 10, 11].

Since irisin was identified as an important molecule in attenuating metabolic disorders, suppressing inflammation and anti-oxidant stress, its functional role in modulating cardiac ischemia and reperfusion remain not clear. Whereas, **Kuloglu et al.** [12] reported that lower plasma level of irisin in patients with acute myocardial infarction (AMI) was shown to be protective. However, some investigators suggested that irisin exerts a possible cardioprotective role [3, 4].

Nevertheless, it is critical to define whether irisin could generate a protective effect against myocardial ischemia and reperfusion injury, which could be developed as a novel strategy in the treatment of cardiovascular diseases.

On the face of this controversy, this study was designed to explore possible effect of irisin on experimentally induced ischemia-reperfusion injury in isolated heart of adult male albino rats and try to clarify the possible mechanisms by which irisin produce its cardioprotective effect.

II. MATERIAL AND METHODS

ANIMALS

A total number of thirty sex adult male albino rats of the local strain weighing 200 - 250 gm were used. All the animals were bred in the animal house of Faculty of Veterinary Medicine Zagazig University. Animals had free access to water, kept at room temperature and were maintained on normal light/dark cycle. The rats were accommodated to animal house conditions for two weeks before the experiments going on and all investigations were conducted in accordance with the guiding principles for the care and use of research animals and

were approved by the Institutional Research Board. Rats were divided into three equal groups (n=12):

Group I: Ischemia-reperfusion (I/R) group, in which isolated hearts were stabilized then subjected to (I/R) protocol, where the hearts were subjected to 30 min. of global no flow ischemia followed by 60 min. of reperfusion. [13].

Group II: Irisin pre-conditioning group, in which irisin (Sigma) (5 ng/ ml) [4,13] was infused for 20 min. before hearts were subjected to the ischemia –reperfusion protocol.

Group III: Irisin post-conditioning group, in which irisin (5 ng/ ml) [4,13]was infused for 20 min. at the beginning of 60 min. reperfusion in the course of ischemia –reperfusion protocol

METHODS

A- Isolated Heart Preparation

rats were anesthetized with urethane (ethyl carbamate) 25% freshly prepared solution in a dose of 1.75- 2 gm/kg injected intraperitoneally [14]. After stabilization of anaesthesia, the animal was placed on aboard in the supine position .The four limbs were extended and fixed to the sides of the board. A midline longitudinal incision started just below the neck and extended to the sternum, the hearts were rapidly excisedand placed in ice-cold Krebs-Henseleit buffer. Then the hearts were suspended to a Langendorff apparatus and retrogradely perfused via aorta at a constant flow rate (12 ml/minute) with the Krebs-Henseleit buffer in a non-recirculating way [15,16].

Left ventricular pressure (LVP) was measured with a pressure transducer connected via a catheter to a latex balloon placed in the left ventricle through the left atrium. The balloon was filled with water to achieve a left ventricular end diastolic pressure (LVEDP) of about 5 mmHg. [15,16].

B-Experimental protocol

Ischaemia- reperfusion protocol

After preparation of the recording system, each heart was allowed to stabilize for 40 min and perfused by Krebs-Henseleit solution in the langendorff apparatus at a constant flow rate of 12 ml/min (which was adjusted with a constant-flow perfusion pump) and temperature of 37°C; at this time, baseline parameters were recorded.

After stabilization, hearts were randomly assigned to one of the groups described above and subjected to 30 min of global, no-flow ischemia (by arresting the perfusion pump), during ischemia the hearts were maintained at 37°C by the surrounding medium. This ischemia was followed by 60 minutes of reperfusion [17,18].

Cardiac function assessment

Using Power Lab 4/3 with bridge amplifier, signals were analyzed by Lab Chart Pro software. Heart rate and LVP were measured and the maximum rates of positive and negative changes in LVP (±dP/dt) were calculated throughout the entire time course of reperfusion to get the following cardiac

performance indicators: Left ventricular pressure (LVP), the maximal values of the first derivative of LVP, [+(LVdP/dt) max, mmHg/s], which indicates the maximal rate of left ventricular contraction [17,19,20,21], the maximal rate of left ventricular pressure decline of LVP [-LVdP/dt) max, mmHg/s)], which indicates the maximal rate of left ventricular relaxation [17,19,20,21] and Hear rate (HR) (beat/min.).

Determination of myocardial infarction area using Nitro blue tetrazolium stain (photos 1, 2, 3): to obtain infarct areas, hearts were rapidly removed from the perfusion apparatus at the end of reperfusion, and the left ventricle was dissected into 2 to 3 mm circumferential slices. After 20 min of incubation at 37°C in 0.1 % solution of Nitro blue tetrazolium in phosphate buffer, unstained necrotic tissue was carefully separated from stained viable tissue. The weights of the necrotic and non-necrotic tissues were then determined, and the necrotic mass was expressed as a percentage of total left ventricular mass, including the septum [17, 19, 20, 22].

C- Biochemical analysis

The reperfusion fluid for each rat was collected throughout the total reperfusion period (60 minutes) and stored at -20 c in a dark container until assaying of the followings:

- Lactate dehydrogenase (LDH), which was measured spectrophotometrically using Vitro Scient, Egypt kits according to the method described by **Moss et al** [14].

- Creatine kinase–MB (CK-MB), which was measured spectrophotometrically using Pointe Scientific, Inc. USA kits according to the method described by [37] and modified by **Rosaki**[23]and Szasz et al [24].

- Super oxide dismutase (SOD), which was assayed by a modified spectrophotometric assay using Bio diagnostic, Egypt kits according to the method of **Kakker et al** [25].

- C- reactive protein (CRP) was measured by immuno-enzymometric assay using Monobind, Inc. Lake Forest, Ca 92630, USA kits according to **Ridker et al** [26].

- The rest of the hearts (after dissection of the left ventricle for staining) were frozen and stored at -20C until analysis of malondialdehyde (MDA) by a spectrophotometer at 534 nm using Bio diagnostic, Egypt kits [27].

STATISTICAL ANALYSIS

The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA). One way analysis of variance (ANOVA) was done followed by LSD test and P value <0.05 was considered statistically significant.

III. RESULTS

Table 1 shows effect of ischaemia reperfusion, application of irisin pre ischemic (5 ng/ ml) and irisin post ischemic (5 ng/ ml) on all measured parameters in the three studied groups.

Table 1: Effect of ischemia-reperfusion, application of irisin pre ischemic (5 ng/ ml) and irisin post ischemic (5 ng/ ml) on all measured parameters in the three studied groups

parameter	(n=12)	Group I (I/R)	Group II (Pre-conditioning irisin + I/R)	Group III (I/R + irisin Post-conditioning
Left ventricular pressure (mmHg)	$\bar{x} \pm SD$	24.88 ± 5.04	26 ± 5.02	31.56 ± 9.04
	p		NS a , c	P < 0.05 a,, b
+ max (LV dp/dt)	$\bar{x} \pm SD$	893.67 ± 251.94	926 ± 225.1	1275 ± 288.9

(mmHg/s)	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
- max (LV dp/dt) (mmHg/s)	$\overline{x} \pm SD$	473.17 ± 111.6	498.83±98.84	637.5±93.76
	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
Heart rate (HR) (beat/min)	$\overline{x} \pm SD$	97.6±33.2	99.5±28.6	137±27
	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
Lactate dehydrogenase (LDH) (IU/L)	$\overline{x} \pm SD$	264.9±100.85	240±83.3	137.4±59.55
	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
Creatine kinase-MB (CK- MB) (IU/L)	$\overline{x} \pm SD$	646.7±200.3	605.8±167.93	417.6±107.4
	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
Superoxide dismutase (SOD) (IU/L)	$\overline{x} \pm SD$	34.31±17.08	34.35±15.23	54.33±15.15
	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
Malondialdhyde (MDA) (nmol/gm tissue protein)	$\overline{x} \pm SD$	7.62±1.85	7.43±1.72	4.66±2.35
	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
C-reactive protein (CRP) (µg/L)	$\overline{x} \pm SD$	330.83±93.81	311.67±77.14	219.33±60.76
	P		NS [a] , P<0.01 [c]	P<0.01 [a, b]
% of wt of necrotic tissue to LV mass	$\overline{x} \pm SD$	57.2±13.69	56±9.89	45.2±1007
	p		NS [a] , P<0.01 [c]	P<0.01 [a, b]

Left ventricular pressure (LVP)

In group I (I/R) LVP was found to range from 18.9 to 32.8 (mmHg) with mean ± SD (24.88 ± 5.04) (mmHg).In group II (Irisin Pre-conditioning) LVP was found to range from 20.3 to 37.2 (mmHg) with mean ± SD (26 ± 5.02) (mmHg). The results showed no significant change (P>0.05) when compared to that of group I. In group III (Irisin Post-conditioning) LVP was found to range from 20.5 to 45.8 (mmHg) with mean ± SD (31.56 ± 6.04) (mmHg) and there was a significant increase (P<0.05) when compared to that of group I.

+ max (LV dp/dt)

In group I (I/R) + max (LV dP/dt) was found to range from 601 to 1324 (mmHg) with mean ± SD (893.67 ± 251.94) (mmHg/s). In group II (Irisin Pre-conditioning) + max (LV dP/dt) was found to range from 670 to 1325 (mmHg/s) with mean ± SD (926 ± 225.1) (mmHg/s). There was no significant change (P > 0.05) when compared to that of group I.In group III (Irisin Post-conditioning) + max (LV dP/dt) was found to range from 793 to 1706 (mmHg/s) with mean ± SD (1275 ± 288.9) (mmHg/s) and there was a significant increase (P < 0.01) when compared to that of group I and group II.

- max (LV dp/dt)

In group I (I/R) - max (LV dP/dt) was found to range from 334 to 648 (mmHg/s) with mean ± SD (473.17 ± 111.6) (mmHg/s).

In group II (Irisin Pre-conditioning) - max (LV dP/dt) was found to range from 349 to 679 (mmHg/s) with mean ± SD (498.83 ± 98.84) (mmHg/s).There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) - max (LV dP/dt) was found to range from 467 to 762 (mmHg/s) with mean ± SD (637.5 ± 93.76) (mmHg/s) and there was a significant increase (P < 0.01) when compared to that of group I and group II.

Heart rate (HR)

In group I (I/R) HR was found to range from 61 to 148 (beat/min) with mean ± SD (97.6 ± 3302) (beat/min). In group II (Irisin Pre-conditioning) HR was found to range from 68 to 153 (beat/min) with mean ± SD (99.5 ± 28.6) (beat/min). There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) HR was found to range from 85 to 168 (beat/min) with mean ± SD (137 ± 27)

(beat/min) and there was a significant increase (P < 0.01) when compared to that of group I and group II.

Lactate dehydrogenase

In group I (I/R) (LDH) level was found to range from 137 to 406 (IU/L) with mean ± SD (264.9 ± 100.85) (IU/L). In group II (Irisin Pre-conditioning) (LDH) level was found to range from 18115 to 380 (IU/L) with mean ± SD (240 ± 83.3) (IU/L). There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) (LDH) level was found to range from 70 to 264 (IU/L) with mean ± SD (137.4 ± 59.55) (IU/L) and there was a significant decrease (P < 0.01) when compared to that of group I and group II.

Creatine kinase-MB (CK- MB)

In group I (I/R) (CK- MB) (IU/L) was found to range from 379 to 918 (IU/L) with mean ± SD (646.7 ± 200.3) (IU/L). In group II (Irisin Pre-conditioning) (CK- MB) (IU/L) was found to range from 359 to 869 (IU/L) with mean ± SD (605.8 ± 167.93) (IU/L).There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) (CK- MB) was found to range from 310 to 664 (IU/L) with mean ± SD (417.6 ± 107.4)(IU/L) and there was a significant decrease (P < 0.01) when compared to that of group I and group II.

Superoxide dismutase (SOD)

In group I (I/R) SOD was found to range from 18.2 to 61.5 (IU/L) with mean ± SD (34.31 ± 17.08) (IU/L) In group II (Irisin Pre-conditioning) SOD was found to range from 19.2 to 63.7 (IU/L) with mean ± SD (34.35 ± 15.23) (IU/L).There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) SOD was found to range from 25.9 to 69 (IU/L) with mean ± SD (54.33 ± 15.15) (IU/L) and there was a significant increase (P < 0.01) when compared to that of group I and group II.

Malondialdhyde (MDA)

In group I (I/R) MDA was found to range from 4.9 to 9.8 (nmol/gm tissue protein) with mean ± SD (7.62 ± 1.85) (nmol/gm tissue protein). In group II (Irisin Pre-conditioning) MDA was found to range from 3.8 to 9.3 (nmol/gm tissue protein) with mean ± SD (7.43 ± 1.72) (nmol/gm tissue protein). There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) MDA was found to range from 1.5 to 8.6

(nmol/gm tissue protein) with mean ± SD (4.66 ± 2.35) (nmol/gm tissue protein) and there was a significant decrease (P < 0.01) when compared to that of group I and group II.
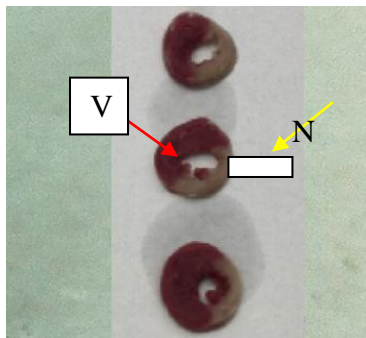
C-reactive protein (CRP)

In group I (I/R) CRP was found to range from 198 to 467 (µg/L) with mean ± SD (330.83 ± 93.81) (µg/L). In group II (Irisin Pre-conditioning) CRP was found to range from 189 to 456 (µg/L) with mean ± SD (311.67 ± 77.14) (µg/L). There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) CRP was found to range from 150 to 345 (µg/L) with mean ± SD (219.33 ± 60.76) (µg/L) and there was a significant decrease (P < 0.01) when compared to that of group I and group II.
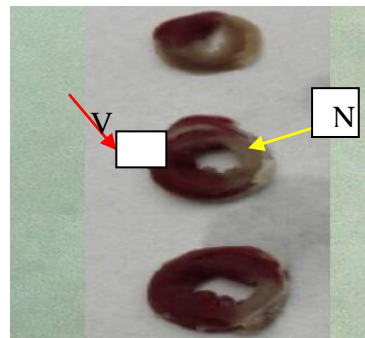
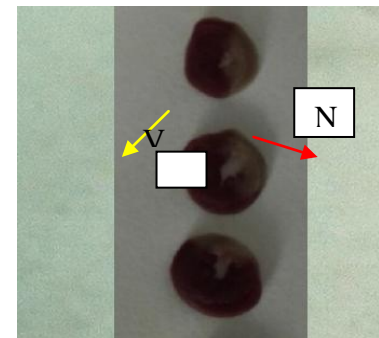a = VS group I   b = VS group II   c = VS group III
NS = non significant (P > 0.05)

% of wt of necrotic tissue to LV mass

In group I (I/R) % of wt of necrotic tissue to LV mass was found to range from 26 to 70 % with mean ± SD (57.2 ± 13.69) %. In group II (Irisin Pre-conditioning) % of wt of necrotic tissue to LV mass was found to range from 34 to 70 % with mean ± SD (56 ± 9.89) %. There was no significant change (P > 0.05) when compared to that of group I. In group III (Irisin Post-conditioning) % of wt of necrotic tissue to LV mass was found to range from 30 to 59 % with mean ± SD (45 ± 10.07) % and there was a significant decrease (P < 0.01) when compared to that of group I and group II.

Picture (1): Left ventricle slices stained by nitro blue tetrazolium stain in group I (I/R)

Picture (2): Left ventricle slices stained by nitro blue tetrazolium stain in group II (pre conditioning group)

Picture (3): Left ventricle slices stained by nitro blue tetrazolium stain in group III (Post conditioning group)

*V: Stained viable tissue   ***N:** Unstained necrotic tissue

IV. DISCUSSION

Cardiac ischemia followed by reperfusion still remains a serious problem in clinical procedures, such as thrombolysis, percutaneous transluminal coronary angioplasty and coronary bypass surgery which are the general treatment strategies of cardiovascular events [11, 28].

Although prompt myocardial ischemia/reperfusion (I/R) might be effective against myocardial impairment, but it also will cause secondary injury via mitochondrial dysfunction and overproduction of pro-inflammatory mediators and reactive oxygen radicals that can provoke further myocardial damage [1].

Ischemic post-conditioning (IPC) is a procedure that has been studied in both animals and humans as a way of protecting the heart and brain cells from reperfusion injury after prolonged ischemia [1]. Therefore, novel treatment strategies are required to attenuate I/R injury and improve clinical outcome, and more feasible and effective drugs are needed in clinical IPC.

It is well known that exercise improve life & reduces the risk of hospital admissions in patients with heart failure & ischemic heart disease by decreasing neurohormonal activity and improving cardiorespiratory fitness [11, 29]. In addition, it also attenuates the progressive cardiac remodelling and decrease in ejection fraction post MI [30]. However, the mechanisms by which exercise triggers these beneficial effects on the heart are still unclear [11, 31].

Irisin, a novel myokine, was discovered in a screen for factors secreted by muscle in response to exercise by *Bostrom et al.* [5] in **2012**. Irisin was first reported to protect against diet-induced weight gain through the browning of white adipose tissue (WAT) and the consequent increase in energy expenditure [5].

The beneficial effects of irisin on the metabolic syndromes produced not only though browning of white adipose tissue and increasing the energy expenditure, but also suppressing inflammation and oxidative stress were reported by many different studies [9, 10, 11, 32].

Therefore, studying irisin could be a therapeutic approach against metabolic and other disorders that are improved with exercise like cardiovascular diseases. However, whether irisin protects the heart against myocardial ischemia and reperfusion (I/R) injury remains unknown [33].

Therefore, this study was designed to explore the effect of irisin on ischaemia-reperfusion injury in isolated heart preparation of male albino rats. The rats were divided into three groups, group (I) ischaemia-reperfusion (I/R); where the ischaemia-reperfusion protocol was applied. Group (II) (pre-conditioning group); in which irisin was applied 20 minutes before I/R protocol. Group (III) (post-conditioning group); after I/R -protocol, irisin was applied to hearts for 20 minutes at the beginning of the reperfusion period.

In this study, it was found that in post conditioning group (group III) there was a significant increase of the studied cardiac parameters; irisin significantly increased LVP, +max (dp/dt), -max (dp/dt) and heart rate when compared to that of control group (group I).

These results are in agreement with **Zhao et al.** [4] who found that irisin generates protective effects on cardiomyocytes exposed to ischemia-reperfusion, by improving cardiac function & post-ischemic ventricular functional recovery, attenuating apoptosis, and improving mitochondrial function.

In the same context, **Li et al.** [31] reported that irisin reduces ischemia-induced neuronal injury and contributes to the neuroprotective effect of physical exercise against cerebral

ischemia, suggesting that irisin may be a factor linking metabolism and cardio-cerebrovascular diseases.

In contrast to our results, **Kuloglu et al.** [12] reported that the decrease in serum irisin & irisin expression in the cardiac muscle after acute myocardial infarction, protects the myocardial cells by saving the extra energy that would have to be given to ischemic mycardiocytes, achieved by inhibiting ATP loss [34].

This discrepancy could be explained by different timing, as the protective role of irisin is mainly during reperfusion rather than during ischemia [35, 36].

Furthermore, in post-conditioning group (group III); irisin significantly decreased the percentage of necrotic tissue to the whole LV mass. This is in agreement with other studies that found a significant reduction in myocardial infarct size and improved post-ischemic functional recovery [3, 4].

In addition, in post conditioning group, irisin caused a significant decrease in LDH and CK-MB levels when compared to that of group I and group II. LDH and CK-MB are well known markers of myocardial damage in which the cardiac cell membrane becomes permeable or may rupture resulting in leakage of these enzymes [37]. So, irisin could decrease the cardiac damage and these results were agreed with **Wang et al.** [3] who found a significant decrease in lactate dehydrogenase (LDH) leakage and apoptotic cardiomyocytes.

This study tries to explore the precise mechanism by which irisin induced a cardioprotective role. The first possible explanation is the anti-oxidant effect of irisin during reperfusion, as the present study found that irisin caused a significant increase of superoxide dismutase (SOD) and a significant decrease of malondialdehyde (MDA) in post conditioning group (group III), when compared to that of group I and group II, which are good markers of oxidative stress; and it is well known that protective effects against myocardial I/R injury are associated with increased anti-oxidant function [38]. These results agreed with **Wang et al.** [3] who reported increased SOD levels following irisin treatment compared to I/R control mice.

SOD is an oxygen radical scavenger; plays an important role in cellular defense, which can protect cells against oxidative damage caused by oxidant radicals such as superoxide anion and hydroxyl radical, which are produced within the first few minutes of reflow, and play a significant role in the development of reperfusion injury [39,40,41].

These free radicals play a role in the pathogenesis of AMI and are capable of reacting with unsaturated lipids and initiating reactions of lipid peroxidation that produce MDA as a marker of oxidative damage [42]. This anti-oxidant role of irisin in myocardial ischemia-reperfusion injury is supported by other investigators who reported an anti oxidant function of irisin against myocardial I/R injury [3, 4]. Also anti-oxidant effect of irisin in other tissues as lung & brain was suggested by other researchers [11, 43].

Therefore, it could be concluded that the increase in SOD following irisin-treatment suggests that the increased anti-oxidant role is responsible for the function of irisin-elicited protection.

The second possible mechanism of irisin cardioprotective effect is the anti-inflamatory role of irisin during reperfusion. Whereas, in the present study, irisin significantly decreased CRP level in post-conditioning group (group III) when compared to that of group I and group II, indicating that irisin has anti-inflammatory property which is protective against cardiac I/R. This finding is in line with other investigators who reported the beneficial effects of irisin on metabolism syndromes, through suppressing inflammation [9, 32, 44, 45, 46].

This anti-infamatory effect of irisin in myocardial ischemia / reperfusion injury is supported by other investigators who found that irisin significantly decrease the CRP and oxidizing free radicals in ischemia/reperfusion injury in lung [43]. Moreover, it was reported that irisin ameliorate pulmonary inflammation in acute lung injury [32].

Notably, oxidative stress & inflammatory mediators released during ischemia / reperfusion injury alter mitochondrial membrane permeability, which is ordinarily impermeable to most ions and metabolites. This prime a non-specific pore in the inner mitochondrial membrane called the mitochondrial permeability transition pore (MPTP) [47].

At the time of reperfusion, this pore opens and allows proteins to move freely across the membrane, with two important consequences: First, the increased osmotic load induced by the flow of constituents into the mitochondrial body causes the mitochondrion to swell and ultimately forces rupture of the outer membrane. The subsequent release of mitochondrial proteins stimulates apoptosis [47, 48]. Second, disruption of the ionic gradient across the mitochondrial membrane uncouples oxidative phosphorylation and results in the hydrolyzing of ATP instead of its synthesis. The subsequent fall in ATP levels further disrupts ionic and metabolic homeostasis and leads to the activation of degradative enzymes [47, 49].

Substantially, the mitochondrial permeability transition pore plays a critical role in the pathogenesis of myocardial ischemia-reperfusion injury [47, 5,]. The cardioprotective effect of inhibiting mPTP and suppressing mitochondrial apoptosis was reported by many previous studies [47, 51, 52].

Moreover, **Zeng and Chen** [43] **reported that** irisin protect the lung from ischemia/reperfusion injury, via reducing the inflammatory and superoxide factors and ameliorating the mitochondrial dysfunction.

Therefore, the cardioprotective role of irisin could be suggested by suppression of mitochondrial apoptosis and inhibition of mitochondrial permeability transition pore opening, increasing the resistance of cardiomyoctes exposed to ischemia-reperfusion, [3,4].

A third possible mechanism by which irisin play a cardioprotective effect, is the anti-apoptosis role of irisin which reported by other researchers who suggested that the protective effects of irisin associated with actvation of p38 phosphorylation in the myocardium, which indicates that anti-apoptosis plays an essential role for irisin-induced protection [3, 32]. However, unfortunately it was not estimated in the present study.

The p38 Mitogen-activated protein kinase (MAPK) is a family of serine/threonine protein kinases that plays an important role in cellular responses to external stress signaling and also functions in many cellular processes, including inflammation, cell differentiation, cell growth and death [35,53,54]. Activation of p38 is associated with cardiac protection [55, 56, 57].

Interstingly , in the presnt study, it was found that in pre conditioning group (group II); irisin produced no significant effect on cardiac function parameters; LVP, + max (dp/dt), -

max (dp/dt) and heart rate and the infracted area size, and level of SOD, CRP & LDH when compared with control group (group I) indicating that irisin has no role in preconditioned ischaemic heart.

It is important to distinguish between the effect of ischemia on the heart and that of reperfusion. Prior to cell death there is a period during which the ischemic myocyte is viable, but vulnerable to further injury if blood flow is restored (ie, reperfusion injury). During this period, the reintroduction of oxygen and energy into an abnormal cellular environment triggers additional events that produce further myocyte damage [36].

This effect has been attributed to production of excessive reactive oxygen radicals [58], which are an important mechanism of reperfusion injury, when molecular oxygen is reintroduced into a previously ischemic myocardium, undergoes a sequential reduction leading to the formation of oxygen free radicals and the influx of the inflammatory cells, leading to rupture of the cell membrane [2].

These deleterious effects were counteracted by giving irisin during reperfusion as a post-conditioning factor (group III), but this was not the case if given before ischemia (group II). However, the antioxidant and anti inflammatory role of irisin in preconditioning group was not sufficient to protect the heart and improve its functions. This may be due to the timing of administration being given before ischemia not during the reperfusion period in the course of myocardial I/R [35, 36].

Therefore, it could be concluded that, irisin postconditioning in group III produced a cardioprotective effect against ischemia / reperfusion injury, as indicated by an improvement in post-ischemic ventricular function and reduction of infarct size; via anti-inflammatory and anti-oxidant effects against free radicals formed during reperfusion.

Acknowledgment

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