EFFECT OF NESFATIN-1 ON ISCHAEMIA-REPERFUSION INJURY IN ISOLATED HEART OF ALBINO RATS

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ABSTRACT

Background: Nesfatin-1 is a hypothalamic neuropeptide which is involved in control of food intake and glucose homeostasis. Lower plasma level of Nesfatin-1 in patients with acute myocardial infarction (AMI) was reported.

Objective: This study was designed to explore possible effect of Nesfatin-1 on ischemia-reperfusion injury in isolated heart of adult male albino rats and to explain the possible involved mechanisms, in a trial to clarify Nesfatin-1 expected cardioprotective effect.

Design: This study was carried out on eighteen adult male albino rats which were divided equally (n=6) into 3 groups: Group I (ischemia-reperfusion I/R group); hearts were stabilized then subjected to (I/R) protocol, Group II (Nesfatin-1 pre-conditioning group); Nesfatin-1 was infused for 20 minutes before hearts were subjected to ischemia and Group III (Nesfatin-1 post-conditioning group); Nesfatin-1 was infused for 20 minutes at the beginning of 120 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). Nesfatin-1 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, Nesfatin-1 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 except for significant increase in SOD level and significant decrease in CRP level.

Conclusion: Nesfatin-1 protects 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 Nesfatin-1 among the strategies for management of cardiac infarction during reperfusion.

Key words: Nesfatin-1, cardiac ischemia-reperfusion, oxidative stress, inflammation.

INTRODUCTION

Nesfatin-1 is a hypothalamic neuropeptide composed of 82 amino acids with a molecular weight of 9.8 kDa, it is derived from the larger protein nucleobindin-2 (NUCB2) [1,2,3].

Nesfatin-1 gene is expressed in the hypothalamic nuclei that involved in feeding behavior, food intake, body weight control and glucose homeostasis [4,5]. The mRNA expression of Nesfatin-1 is significantly decreased by fasting and significantly increased in the hypothalamus on refeeding, suggesting that Nesfatin-1 is an anorexigenic hormone [6].

In addition to regulating appetite [5], Nesfatin-1 plays key roles in energy homeostasis and metabolism [7,8]. In the last few years, Nesfatin-1 was considered an adipocytokine [9]with a close relationship found between this peptide and diabetes, as its plasma levels were reduced in type II diabetic patients [10]. In fact, Nesfatin-1 elicits anti-hyperglycemic effects [11] and its levels in serum of obese individuals were decreased significantly as the degree of obesity increased (from obese to morbid obese) [12].

Interestingly, rat heart constitutively expresses both the precursor NUCB2 and the protein Nesfatin-1[1,13]. This expression is

comparable to that detected in the brain, which is known to be Nesfatin-1-producing organ [14]. Accordingly, the cardiac tissue appears to be a source for Nesfatin-1, which may act in a paracrine/autocrine manner on the heart itself and this expression of Nesfatin-1 and the precursor NUCB2 allows the inclusion of the peptide in the growing list of cardiac hormones [15]. Furthermore, lower plasma level of Nesfatin-1 in patients with acute myocardial infarction (AMI) was reported [16].

In patients with AMI, the treatment of choice for reducing acute myocardial ischemic injury and limiting MI size is and effective myocardial reperfusion using either thombolytic therapy or primary percutaneous coronary intervention (PPCI). However, the process of reperfusion can itself induce cardiomyocyte death, known as myocardial reperfusion injury, for which there is still no effective therapy [17]. Moreover, it is possible to say that oxidative and inflammatory response activation are extremely important in ischemia-reperfusion (MI/R)injury phenomenon [18].

It is worth saying that Nesfatin-1 could serve a neuro and gastroprotective function by supporting the balance in oxidant and antioxidant systems and by affecting inflammatory profile [19,20,21] ,So the reduced expression of Nesfatin-1 may play a role in the pathogenesis of MI/R injury [19,20]. Moreover, administration of exogenous Nesfatin-1 may play a cardioprotective effect in those patients.

The aim of this study is to explore the possible effect of Nesfatin-1 on ischemia-reperfusion injury in isolated heart of adult male albino rats and to explain the possible involved mechanisms, in a trial to clarify its expected cardioprotective effect.

MATERIAL AND METHODS

Animals

A total number of eighteen 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 a 12 h 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=6):

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 120 min. of reperfusion.

Group II: Nesfatin-1 pre-conditioning group, in which Nesfatin-1(Sigma) (100 pmol /L) [15]was infused for 20 min. before hearts were subjected to the ischemia –reperfusion protocol.

Group III: Nesfatin-1 post-conditioning group, in which Nesfatin-1(100 pmol /L) [15]was infused for 20 min. at the beginning of 120 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 [29].-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 [22,23].

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. [22,23].

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 120 minutes of reperfusion [15,24].

-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 [15,25,26,27], the maximal rate of left ventricular pressure decline of LVP [-LVdP/dt) max, mmHg/s)], which indicates the maximal rate of left ventricular relaxation [15,25,26,27] 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 37ºC 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 [15,23,24,28].

C- Biochemical analysis:

The reperfusion fluid for each rat was collected throughout the total reperfusion period (120 minutes) and stored at -20 c in 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** [29].
- Creatine kinase–MB (CK-MB), which was measured Spectrophotometrically using Pointe Scientific, Inc. USA kits according to the method described by Oliver, 1955 and modified by **Rosaki[30]and Szasz et al** [31].
-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** [32].
-C- reactive protein (CRP) was measured by Immuno-enzymometric assay using Monobind, Inc. Lake Forest, Ca 92630, USA kits according to **Ridker et al** [33].
-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 534nm using Bio diagnostic, Egypt kits [34].

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.

RESULTS

Table 1 shows effect of ischaemia reperfusion, application of Nesfatin-1 pre ischemic (100 pmol/L) and Nesfatin-1 post ischemic (100 pmol/L) on all measured parameters in the three studied groups.

Left ventricular pressure (LVP)

In group I (I/R) LVP was found to range from 18.4 to 33.6 (mmHg) with mean ± SD (25.6 ± 5.8) (mmHg).In group II (Nesfatin-1 Pre-conditioning) LVP was found to range from 22.2 to 38.7 (mmHg) with mean ± SD (28.2 ± 5.8) (mmHg). The results showed no significant change (P>0.05) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) LVP was found to range from 62.6 to 77.4(mmHg) with mean ± SD (69.7 ± 5.3) (mmHg) and there was a

significant increase (P<0.001) 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 904 to 1394 (mmHg) with mean ± SD (119.3 ± 203.7) (mmHg/s).In group II (Nesfatin-1 Pre-conditioning) + max (LV dP/dt) was found to range from 1109 to 1704 (mmHg/s) with mean ± SD (1412.0 ± 240.6) (mmHg/s). The results showed no significant change (P>0.05) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) + max (LV dP/dt) was found to range from 2061 to 2673 (mmHg/s) with mean ± SD (2361.2 ± 228.8) (mmHg/s) and there was a significant increase (P<0.001) 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 611 to 943 (mmHg/s) with mean ± SD (776.5±122.8) (mmHg/s).

In group II (Nesfatin-1 Pre-conditioning) - max (LV dP/dt) was found to range from 854 to 1194 (mmHg/s) with mean ± SD (982.8±117.9) (mmHg/s). The results showed no significant change (P>0.05) when compared to that of group .In group III (Nesfatin-1 Post-conditioning) - max (LV dP/dt) was found to range from 1108 to 1397(mmHg/s) with mean ± SD (1263.8±111.1) (mmHg/s) and there was a significant increase (P<0.001) when compared to that of group I and group II.

Heart rate (HR)

In group I (I/R) HR was found to range from 59.7 to 75.13 (beat/min) with mean ± SD (66.9±6.1) (beat/min).In group II (Nesfatin-1 Pre-conditioning) HR was found to range from 73.3 to 89.5 (beat/min) with mean ± SD (79.66±5.9) (beat/min). The results showed no significant change (P>0.05) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) HR was found to range from 90.2 to 105.3(beat/min) with mean ± SD (98.1±5.6) (beat/min) and there was a significant increase (P<0.001) when compared to that of group I and group II.

Lactate dehydrogenase

In group I (I/R) (LDH) level was found to range from 168 to 217(IU/L) with mean ± SD (199.8±21.8) (IU/L).In group II (Nesfatin-1 Pre-conditioning) (LDH) level was found to range from 180 to 205(IU/L) with mean ± SD (195.3±8.8) (IU/L). The results showed no significant change (P>0.05) when compared to that of group I.In group III (Nesfatin-1 Post-conditioning) (LDH) level was found to range from 40 to 86(IU/L) with mean ± SD (59.5±18.7) (IU/L) and there was a significant increase (P<0.001) 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 557to 813 (IU/L) with mean ± SD (707.2 ± 110.5) (IU/L). In group II (Nesfatin-1 Pre-conditioning) (CK- MB) (IU/L) was found to range from 541 to 802 (IU/L) with mean ± SD (679 ± 114.3) (IU/L) .The results showed no significant change (P>0.05) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) (CK-MB) was found to range from 114 to 221 (IU/L) with mean ± SD (153.5 ± 37.2) (IU/L) and there was a significant increase (P<0.001) when compared to that of group I and group II.

Superoxide dismutase (SOD)

In group I (I/R) SOD was found to range from 15.2 to 22.18 (IU/L) with mean ± SD (18.7 ± 2.5) (IU/L). In group II (Nesfatin-1 Pre-conditioning) SOD was found to range from 38.2 to 55.1 (IU/L) with mean ± SD (47.6 ± 6.2) (IU/L).The results showed a significant increase (P<0.001) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) SOD was found to range from 109.6 to 118 (IU/L) with mean ± SD (113.6 ± 3.1) (IU/L) and there was a significant increase (P<0.001) when compared to that of group I and group II.

Malondialdhyde (MDA)

In group I (I/R) MDA was found to range from 5.58 to 8.17 (nmol/gm tissue protein) with mean ± SD (6.94 ± 1.15) (nmol/gm tissue protein). In group II (Nesfatin-1 Pre-conditioning) MDA was found to range from 4.83 to 8.05 (nmol/gm tissue protein) with mean ± SD (6.5 ± 1.2) (nmol/gm tissue protein). The results showed no significant change (P>0.05) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) MDA was found to range from

1.52 to 3.8 (nmol/gm tissue protein) with mean ± SD (3.04 ± 0.83) (nmol/gm tissue protein) and there was a significant decrease (P<0.001) 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 423to 475 (µg/L) with mean ± SD (447.5±20.8) (µg/L). In group II (Nesfatin-1 Pre-conditioning) CRP was found to range from 289 to 360(µg/L) with mean ± SD (336.0± 25.9) (µg/L). The results showed a significant decrease (P<0.001) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) CRP was found to range from 166 to 195 (µg/L) with mean ± SD (181.8±10.9) (µg/L) and there was a significant decrease (P<0.001) when compared to that of group I and group II.

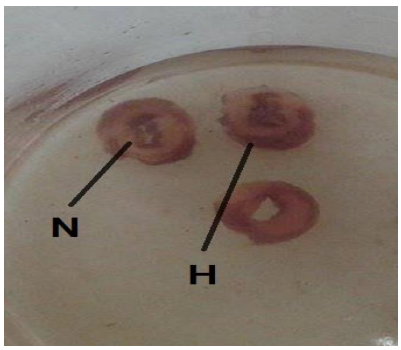
% of wt of necrotic tissue to LV mass

In group I (I/R) % of wt of necrotic tissue to LV mass was found to range from 47 to 60 with mean ± SD (55±4.7). In group II (Nesfatin-1 Pre-conditioning) % of wt of necrotic tissue to LV mass was found to range from 48 to 58 with mean ± SD (52.8±4.1). The results showed no significant change (P>0.05) when compared to that of group I. In group III (Nesfatin-1 Post-conditioning) % of wt of necrotic tissue to LV mass was found to range from 21 to 34 with mean ± SD (26.2±4.9)and there was a significant increase (P<0.001) when compared to that of group I and group II.

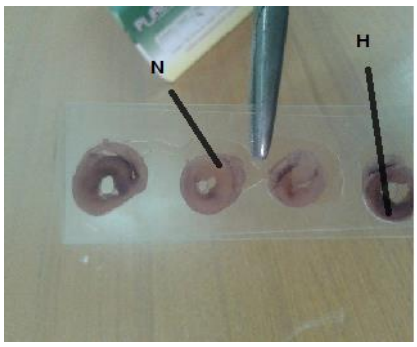
Table 1: Effect of ischaemia reperfusion, application of Nesfatin-1 pre ischemic (100 pmol/L) and Nesfatin-1post ischemic (100 pmol/L) on all measured parameters in the three studied groups

parameter	(n=6)	Group I (I/R)	Group II (Pre-conditioning Nesfatin-1 + I/R)	Group III (I/R + Nesfatin-1 Post-conditioning
Left ventricular pressure (mmHg)	$\overline{X} \pm SD$	25.6 ± 5.8	28.2± 5.8	69.7± 5.3
	p		NS [a] , P < 0.001 [c]	P < 0.001 [a, b]
+ max (LV dp/dt) (mmHg/s)	$\overline{X} \pm SD$	1119.3± 203.7	1412.0± 240.6	2361.2± 228.8
	p		NS a , P < 0.001c	P < 0.001 [a,b]
- max (LV dp/dt) (mmHg/s)	$\overline{X} \pm SD$	776.5± 122.8	982.8± 117.9	1263.8± 111.1
	p		NS [a], P < 0.001 [c]	P < 0.001 [a, b]
Heart rate (HR) (beat/min)	$\overline{X} \pm SD$	66.9±6.1	79.66±5.9	98.1±5.6
	p		NS [a], P < 0.001 [c]	P < 0.001 [a, b]
Lactate dehydrogenase (LDH) (IU/L)	$\overline{X} \pm SD$	199.8±21.8	195.3±8.8	59.5±18.7
	p		NS [a] , P < 0.001 [c]	P < 0.001 [a, b]
Creatine kinase-MB (CK- MB) (IU/L)	$\overline{X} \pm SD$	707.2 ± 110.5	679 ± 114.3	153.5 ± 37.2
	p		NS [a], P < 0.001 [c]	P < 0.001 [a,b]
Superoxide dismutase (SOD) (IU/L)	$\overline{X} \pm SD$	18.7 ± 2.5	47.6 ± 6.2	113.6 ± 3.1
	p		P < 0.001 [a c]	P < 0.001 [a,b]
Malondialdhyde (MDA) (nmol/gm tissue protein)	$\overline{X} \pm SD$	6.94 ± 1.15	6.5 ± 1.2	3.04 ± 0.83
	p		NS [a], P < 0.001 [c]	P < 0.001 [a, b]
C-reactive protein (CRP) (µg/L)	$\overline{X} \pm SD$	447.5±20.8	336.0± 25.9	181.8±10.9
	p		P < 0.001 [a c]	P < 0.001 [a, b]
% of wt of necrotic tissue to LV mass	$\overline{X} \pm SD$	55±4.7	52.8±4.1	26.2±4.9
	p		NS [a], P < 0.001 [c]	P < 0.001 [a, b]

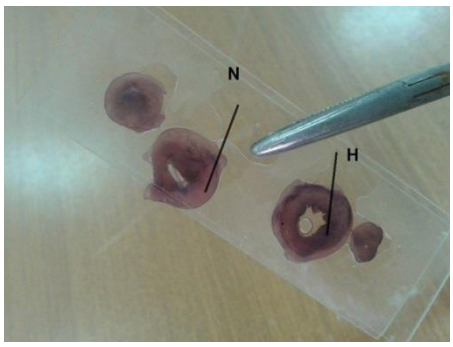
a = VS group I b = VS group II c = VS group III
NS = non signifiant (P > 0.05)



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)

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

DISCUSSION

Cardiac ischemia followed by reperfusion still remains a serious problem in clinical procedures, such as thrombolysis, percutaneous trans-luminal coronary angioplasty and coronary bypass surgery which are the general treatment strategies of cardiovascular events [17].

Nesfatin-1 is an 82 amino-acid hypothalamic neuropeptide reported to decrease food intake [1], besides its expression in central nervous system; it is widely expressed peripherally and considered to be an adipocytokine [13,35,36]. Nesfatin-1 acts as an integral regulator of energy balance, circadian feeding rhythm, and related endocrine functions [37].Its peripheral administration in normal rats decreased blood glucose level [38]. Also, in type II diabetic patients Nesfatin-1 plasma level was reported to be lower than controls [39].

Nesfatin-1 is expressed in the ventricular cardiomyocytes [1,13,40] and low levels were reported in patients with AMI, which may play an important role in the development of AMI [16].

In this study, effect of Nesfatin-1 was investigated 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 Nesfatin-1 was applied20 minutes before I/R protocol. Group (III) (post-conditioning group); after I/R protocol, Nesfatin-1 was applied to hearts for 20 minutes at the beginning of the reperfusion period.

This study provides evidence that Nesfatin-1 possesses cardioprotective properties, as in group III (post conditioning group), there was a significant increase of the studied cardiac parameters. Nesfatin-1 significantly increased LVP, +max (dp/dt), -max (dp/dt) and HR compared to I/R group. This comes in consistence with the study of **Angelone et al.**[15] which stated that there is a dose dependant improvement of LVP, +max(dp/dt) , -max(dp/dt) and HR when Nesfatin-1 was reperfused just after ischaemic period in the first 20 min. of reperfusion and abolished contracture development at the end of reperfusion.

In post conditioning group, Nesfatin-1 caused a significant decrease in LDH and CK-MB levels, which are well known markers of myocardial damage in which the cardiac cell membrane becomes permeable or may rupture resulting in leakage of these enzymes [41].So, Nesfatin-1 could decrease the cardiac damage and this was supported by the decrease in percentage of necrotic tissue to the LV mass in this group. These results were agreed by **Angelone et al.** [15]who found a significant decrease of LDH when isolated hearts were reperfused with Nesfatin-1, as a post conditioning factor. Also, Nesfatin-1 was reported to reduce lactate dehydrogenase (LDH) release in renal I/R [42].

Another finding in the present study is that, Nesfatin-1 caused a significant increase of SOD and a significant decrease of MDA in

post conditioning group (group III), which are good markers of oxidative stress [43].

A landmark study by Bolli et al. [44] showed that potent oxidant radicals such as superoxide anion, hydroxyl radical, and peroxynitrite, are produced within the first few minutes of reflow and play a crucial role in the development of reperfusion injury. These Free radicals play a significant role in the pathogenesis of AMI [45] and are capable of reacting with unsaturated lipids and initiating self perpetuating chain reaction of lipid peroxidation that produce MDA as a marker of oxidative damage [46].

SOD is an oxygen radical scavenger; its localization in mitochondria as well as its inducibility in response to mid injurious stimuli suggests that SOD plays an important role in cellular defense, which can protect cells against oxidative damage by converting superoxide anion radicals that occur in the upper stream of the reactive oxygen metabolism cascade [47]This enzyme plays important roles in oxidative stress during I/R injury [48].

This antioxidant role of Nesfatin- 1 in myocardial ischemia reperfusion injury is supported by other investigators who reported an anti oxidant function of Nesfatin-1 in cases of sub arachnoid heamorrhage of rat brain [19].

Furthermore, Nesfatin-1 significantly decreased CRP level in post conditioning group (group III), indicating that Nesfatin-1 has anti inflammatory property which is protective against cardiac I/R.

This finding is in line with other investigators who concluded that, Nesfatin-1 can be protective for intestinal and renal ischemia reperfusion injury by inhibiting the generation of pro-inflammatory mediators [21].

Also, Nesfatin-1 can alleviate gastric inflammatory damage induced by indomethacin [21]. Moreover, the administration of Nesfatin-1 after head trauma could significantly suppress gene expressions of nuclear factor kappa –B, lessen concentrations of tumor necrosis factor-alpha, interleukin-1beta and interleukin-6 which are important inflammatory mediators[20].

Furthermore, Nesfatin-1 was reported to be a new anti- inflammatory factor in case of systemic inflammation in chronic obstructive pulmonary disease (COPD) [49] and in obstructive sleep apnea syndrome [50].

In addition, in post conditioning group (group III); Nesfatin-1 significantly decreased the percentage of necrotic tissue to the whole LV mass. This is in agree with **Angelone et al** [15] who reported a similar effect when Nesfatin-1 was administered in vitro during the first 20 minutes of reperfusion in case of myocardial I/R.

Thus, it can be concluded that, Nesfatin-1 postconditioning in group III produced a cardioprotective effect via anti-oxidant and anti-inflammatory effects.

The cardioprotective role of Nesfatin- 1in post conditioning group can be explained by many mechanisms reported by other researchers; first, Nesfatin- 1 activates the novel PKC isoform as PKCθ, PKCε and PKCe in ventricular myocytes [39].PKCθ and PKCε are considered the most important isoenzymes protecting the heart from reperfusion injury and preserve systolic function, at least in part, by preventing cardiomyocyte cell death. In addition, PKCe may act on mitochondria to affect cellular survival reducing both necrosis and apoptosis [51,52].

Nesfatin-1 diminished activity of the apoptotic enzyme caspase-3 as well as reduced number of apoptotic neuronal cells in traumatic rat brain tissues [20]and tubular apoptotic cells in renal I/R[42]. This indicates that Nesfatin-1 has anti-apoptotic effects.

Second, in rat ventricular myocytes, the nitric oxide synthase(NOS) produces nitric oxide (NO) which by targeting soluble guanylatecyclase (GC) increases protein kinase G(PKG), which in turn negatively affects contractility by reducing the phosphorylating troponin I [53]. It was reported that Nesfatin-1 can inhibit NO production via impairment of cGMP production [54,55],furthermore, chronic peripheral nesfatin-1 administration can decrease endothelial nitric oxide synthase (eNOS) level at the acute phase of ischemia/reperfusion, this will serve two functions simultaneously; limitation of reperfusion flow and counteraction of cardiac depression produced by NO [56].On the contrary, **Angelone et al** [15] concluded that

NO was not involved in the cardioprotective effect of nesfatin-1 in their study.

Biomolecular studies showed that signal transducer and activator of transcription-3 (STAT3) and Extracellular signal-regulated kinases1/2 (ERK1/2) take part to the Nesfatin-1-mediated post-conditioning [15]. It is known that STAT is an important membrane to-nucleus signaling for many stress responses, which include I/R, oxidative stress, and hypoxia [44,57].It is also a crucial member of protective cascades that, when activated, induces survival signals in the infracted myocardium [58,59].

Notably, in group (II) (pre conditioning group), Nesfatin-1 produced no significant effect on cardiac function parameters; LVP, +max(dp/dt), -max(dp/dt) and HR and the infracted area size, despite, the significant increase in level of SOD and the significant decrease in CRP level in this group, indicating that Nesfatin-1 has a minor role in preconditioned ischaemic heart.

It is important to distinguish between the effects of ischemia and the effects of reperfusion on the heart. Necrosis does not occur during ischemia, but rather during the subsequent reperfusion, and this has been attributed to production of excessive reactive oxygen radicals [60], 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 [61].Those deleterious effects were counteracted by giving Nesfatin-1 during reperfusion as a post-conditioning factor (group III), but this was not the case if given before ischemia (group II).

The resulting decrease in CRP in case of Nesfatin-1 preconditioning (group II) could be attributed to its intrinsic biological properties as an acute-phase reactant in case of ischaemic heart disease. In contrast, none of the other systemic markers of inflammation, whether upstream cytokine mediators other sensitive acute-phase proteins has such characteristics [62].This also explained the sensitivity of this marker to anti-inflammatory effect of Nesfatin-1 as a preconditioning factor. The case may be the same for the increased levels of SOD induced by Nesfatin-1 preconditioning, being the most sensitive anti-oxidant [63].

However, this antioxidant and anti-inflammatory role of Nesfatin-1in 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.

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