Cardioprotective effect of Angiotensin (1-7) on myocardial infarction: Possible role of Nitric oxide and prostaglandins

Zienab Abdullah, Soheir A. Helmy, Hanaa G. Elserougy, Amr M. Abbas, Gehan A. Alwakeel

Department of medical physiology, faculty of medicine, Mansoura University, Egypt

Abstract

Objective: to detect the possible cardioprotective effect of Ang-(1-7) in rat model of MI; Also, possible role of NO and PGs in this probable cardioprotective effect of Ang- (1-7) was studied. **Methods**: Rats were divided randomly into 6 groups (8 rats each): **Group I :** Control group**:**, **Group II:** rats received S.C isoprenaline at a dose of 150 mg/kg/day on two consecutive days with an interval of 24 hours between applications, **Group III:** rats received **Ang (1-7)** (576μg/kg/day) S.C for 6 days after induction of MI **,Group IV:** in which rats received **Ang (1-7)** (576μg/kg/day)+ **L-NAME** in the drinking water (80 mg/l) for 6days after induction of MI **,Group V:** in which rats received **Ang (1-7)** (576μg/kg/day) +**Indomethacin** 5 mg/kg/day IP for 6 days after induction of MI **,Group VI:** in which rats received both **Ang-(1-7)**+ L- NAME and Indomethacin at dose mentioned previously. Biochemical, histopathologial , and ECG changes were studied
Results: ISO –MI group exhibited a significant rise in serum cardiac enzymes and disturbed lipid profile, increased myocardial damage score and Caspase3 expression when it is compared to the normal group ($p<0.001$). ECG changes of rat revealed elevation ST segment, QT interval prolongation, decrease QRS duration and voltage, and accelerated heart rate. Ang-(1-7) caused significant improvement in the studied parameters ,while co-infusion of L-NAME and or indomethacin prevent this effect of Ang-(1-7) .Combined L-NAME and indomethacin produce more deleterious effect than separate administration of them. **Conclusion**: Ang-(1-7) is considered one of the cardioprotective components of RAS .NO and PGs mediate the action of Ang-(1-7) and they may have an additive effect.

Corresponding author: Zienab Abdullah, Physiology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. Tel:+201092364414; E mail: shemesmohamed@yahoo.com

Introduction

Cardiovascular diseases are considered one of the most common causes of mortality and morbidity all over the world (1). Myocardial infarction (MI) is considered among the most serious acute lesion affecting the heart, that cause hundreds of thousands of deaths annually worldwide (2).Thus, the continuous studies about the strategies of therapies that can reduce the occurrence of this pathology is still mandatory (3).

The pathophysiology of MI comprises different mechanisms in which RAS play an effective role. The RAS are more complex than previously known, it has two major types of function, according to the type of peptides produced ; a proliferative &vasoconstrictor one in which the major acting peptide is (Ang II) and an anti-proliferative& vasodilator one in which the major effector is Ang-(1-7) (4).

Since it has been identified, Ang-(1-7) has rapidly gained a great position as one of the remarkable cardioprotective molecules in RAS (5).Ang-(1-7) is an active heptapeptide of the RAS; its physiological role are mostly opposite to that produced by Ang II. It binds to specific G protein coupled receptor named *MAS* receptor (the name is the first 3-surname letters of the patient whose tumor cells were used to identify the gene) (4).

Ang-(1-7) may act either by a direct mechanism on its receptor, or indirectly through bradykinin potentiation (6),-NO and prostaglandin release (7).Also, Ang-(1-7) can inhibit ACE (8),so it was suggested that this heptapeptide could be a target to discover new and innovative cardioprotective drugs (7).

Ang-(1-7) may produce many beneficial functions in the heart, including: vasodilation, inhibition of cell proliferation, prevention of hypertrophy, reducing fibrosis, inhibition of thrombosis, and prevention of arrhythmia (8).

Following activation of *MAS* by Ang-(1-7) in the heart, the signal transduction pathways are not fully characterized, however it may involve release of PGs and or NO release or both (4).

In contrast to many studies that reported a cardioprotective role of Ang-(1–7), it has been reported that Ang-(1–7) infusion resulted in harmful events occurred in the heart of Sprague dawley rats (10). Also, **Zhang et al**. revealed the occurrence of myocardial hypertrophy in rats as a consequence of *MAS* overexpression in cardiomyocytes (11).

Several studies have proved that NO and PGs have an influential role in cardioprotection **(12)**. The concentrations of NO and PGs were elevated in the infarcted portions of the heart **(13).**Cardioprotective effects of NO include decreased apoptosis of cardiomyocytes, decreased oxygen consumption and increased ischemic tolerance. In addition, it has been demonstrated that NO improved contractile function through modulating SR Ca2+-ATPase activity (SERCA), RyR, and L-type Ca^{2+} current **(14)**.

L-arginine analogues are widely used inhibitors of nitric oxide synthase (NOS) activity thus decreasing NO.-In fact, N(ω)-nitro-L-arginine methyl ester (L-NAME) comes at the top of the list of these analogues **(15).**

PGs exert their cardioprotective effect through inhibiting the production of chemokines in human macrophages **(16)**, in addition to inhibition of the production of proinflammatory cytokines**(17),** and adhesion molecules **(18)**.These actions of PGs ensure that PGs play a pivotal role prevention of inflammation-related cardiovascular diseases**(19)**.

Indomethacin has been used as a potent non-steroidal anti-inflammatory drug that acts through inhibition of cyclooxygenase thus preventing the production of prostaglandins from arachidonic acid **(20).**

So the aim of this work was to determine the possible cardioprotective role of Ang-(1-7) in rat model of MI; through studying electrocardiographic, biochemical and histopathological changes in those rats. Also, possible role of NO and PGs in this probable cardioprotective effect of Ang- (1-7) was studied.

Materials and Methods

Chemicals:

Angiotensin Fragment 1-7 acetate salt hydrate, L-NAME hydrochloride, Indomethacin, isoproterenol hydrochloride, Ketamine hydrochloride, Xylazine hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO, USA.

 Animals:

Forty eight adult male SD rats aging 4-6 months, and weighing between 200-250 g were used in the thesis.-Rats were bred and housed in the animal house of the Medical Experimental Research center (MERC), Mansoura University, under standardized environment (12-hrs light/dark cycles and temperature of 24 °C).They were fed a standard chow and had free tap water access. The experimental procedures were approved by Mansoura Faculty of Medicine research ethics committee.

Study groups:

Rats were divided randomly into 6 groups (each contain 8 rats): Group I (Control): normal rats, received normal saline (2ml) S.C on two consecutive days .Group II (ISO- induced MI): in which rats received subcutaneous isoprenaline at a dose of 150 mg/kg/day for 2 successive days with 24 hours interval between the doses(21).Group III (ISO- induced MI + Ang-(1-7)): rats received Ang (1-7) (576µg/kg/day) S.C for 6 days after induction of MI (22).Group IV (ISO- induced MI + Ang-(1-7) + L-NAME): rats received Ang-(1-7) at the same previous dose + L-NAME in the drinking water (80 mg/l) for 6days after induction of MI (22).Group V (ISO- induced MI + Ang-(1-7) +Indomethacin): rats received Ang-(1-7) at the same previous dose + Indomethacin 5 mg/kg/day IP for 6 days after induction of MI(23).Group VI (ISO- induced MI + Ang-(1-7) +Indomethacin+ L NAME): rats received both Ang-(1-7) +L- NAME and indomethacin at the same previous dose (24).

Recording of ECG

For all rats in this study, recording of ECG was done at basal day and repeated at the second and eighth days after MI induction in all experimental rats. Light ether anesthesia was used during ECG recording .biopac student lab system (software BSL 3.7.5), MP45 (data acquisition unit), biopac electrode lead set x2 and disposable vinyl

electrodes, 3 electrodes for each rat were used during ECG recording.

Measurement of cardiac enzymes (CK-MB, AST ,-ALT , and LDH)

Cardiac enzymes were measured using commercially available kits according to the manufacturer's instructions. AST, ALT and CK-MB kits were purchased from bioMérieux Diagnostics, Milan, Italy,-while LDH kits were purchased from Bayer Diagnostics Ltd., Baroda, India.

Assay of lipid profile

The lipid profile measured parameters include triglycerides, cholesterol, HDL-CL, and LDL-CL. They were determined by using available kits according to the manufacturer's instructions. Kits were purchased from Spinreact Company, Sant Esteve de Bas, groupGirona, Spain.

Histopathological examination

The cardiac tissues from all rats were washed with saline immediately and fixation in 10% buffered neutral formalin solution was performed, and then embedded in paraffin. The cardiac tissue was sectioned and stained with haematoxylin and eosin (H&E). The results were classified as no changes; +mild (slight cardiomyocyte damage with mild degree of inflammation); ++moderate (excess cardiomyocyte degeneration in association with diffuse inflammation); +++marked (sever necrosis with extensive inflammation). According to these histopathological changes in myocardium; rats were graded into three distinct groups: group A (with no histopathological change), group B (mild affection) and group C (moderate and/or severe affection).

Immunohistochemical detection of caspase 3

Polyclonal antibody of rabbit was used (RP096) was purchased from Diagnostic BioSystems Owens Drive, Pleasanton, CA.

Computer Assisted digital image analysis (Digital morphometric study): Slides were photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 20 X objective. The result images were analyzed on Intel® Core I3® based computer using VideoTest® Morphology® software (Russia) with a specific built-in routine for area measurement, stain density and descriptive geometrical parameters analysis. Five slides from each case were prepared, five random fields from each slide were analyzed.

Quantification of Caspase 3 (Immunohistostaining)

The software routine of quantification includes:

1: Image acquiring form the camera using a u-tech® frame grabber, *2:* Enhancing color tones of the image depending on the hue of target areas. *3:* Thresholding of the image at the level of the desired hue range to form a binary mask that represents target areas, *4*: Define binary mask as region of interest (ROI). *5:* All results were exported as.XLS.

Statistical analysis

The obtained data were represented as Mean ± SD. Comparison for parametric data was done by analysis of variance (ANOVA) followed by turkey's post hoc analysis. P<0.05 was considered significant

Results

Effect of Ang-(1-7), Ang-(1-7) +L-NAME, Ang-(1-7) +Indomethacin Ang-(1-7) +both(L-NAME &Indomethacin) on cardiac enzymes:

Cardiac enzymes results are presented in table (1). At day 0, cardiac enzymes (CK- MB, LDH, AST, and ALT) were not different between all groups. At day 2, cardiac enzymes were increased significantly in all experimental groups in comparison with normal group (p≤ 0.001). At day 8, these markers were decreased significantly in Ang-(1-7) treated group when compared to ISO group as well as to L-NAME, indomethacin, combined (L-NAME, indomethacin) treated groups (p≤ 0.001).Furthermore cardiac enzymes were increased in a significant value in combined

group in comparison with L-NAME or indomethacin groups (p≤ 0.001).

Effect of Ang-(1-7), Ang-(1-7) +L-NAME, Ang-(1-7) +Indomethacin Ang-(1-7) +both (L-NAME &Indomethacin) on lipid profile:

Lipid profile results are presented in table (2). At day 0, lipid profile results were not different between all groups. At day 2, serum Cholesterol, TG, LDL-CL were increased significantly in all experimental groups when compared with control group (p≤ 0.001), while HDL-CL was decreased significantly in all experimental groups when compared with control group (p≤ 0.001).

Table (1): cardiac enzymes in different studied groups at basal, 2 and 8 days.

	Group Days	NC	ISO-MI	ISO+Ang-(1-7)	ISO+Ang-(1-7)+L-NAME	ISO+Ang-(1-7)+Indomethacin	ISO+Ang-(1-7)+both
CK-MB(U/L)	Basal	20.3±.9	21.07±1.2	21.8±1.7	20.3±1.1	21.5±1.3	21.2±1.3
	Day2	21.7±1.4	212.97±13.57 € A	223.55±5.8 € A	216.87±11.26 € A	212±9.2€ A	217.67±9.8€ A
	Day8	20.8±1.5	185.77±19.3	83.67±7.39 Ω B D E F	203.07±4.69 c	202.4±10.4 c	229.5±10.7CDE
LDH(U/L)	Basal	97.15±4.3	99.34±1.86	101.67±6.56	94.93±5.6	95.63±2.83	95.92±4.02
	Day2	97.47±1.17	140.03±5.11 € A	136.67±8.31 € A	130.08±15.66 € A	133.83±7.28 € A	135.53±7.12 € A
	Day8	98.48±4.01	136.33±4.01	120.87±2.95 Ω B D E F	128.81±10.95 c	130.35±3.24 c	150.45±6.86 CDE
AST(U/L)	Basal	74.67±2.7	77±2.6	81.3±7.8	77.17±4.17	81.34±5.61	80.83±4.96
	Day2	75.66±3.67	120.66±8.64 € A	109.16±3.08 € A	127.85±8.92 € A	129.67 ±2.16 € A	139.53±4.27 € A
	Day8	80.33±5.85	108±2	82.2±3.69 Ω B D E F	115.17±10.74 C	113 ±9.39 C	143.3±4.74 C
ALT(U/L)	Basal	67.7±4.5	68.2±5.49	69.5±6.44	68.33±3.08	70±5.65	67.27±3.89
	Day2	69.5±4.89	100±4.04€ A	99.17±3.6€ A	101±3.89€ A	99.89±3.03€ A	102±4.34€ A
	Day8	69±5.86	98.83±2.71	82.33±1.63 Ω B D E F	99.37±2.84 C	98±4.24 C	101.27±3.58 C

The results were represented as mean ± SD, testes used are One way ANOVA and Turkey's post hoc (p≤ 0.05 is considered), NC = normal group, ISO=Isoprenaline, Both= L-NAME+ INDOMETHACIN, €=significant in comparison to basal group, Ω = significant significant in comparison to 2 days group, # = significant significant in comparison to 8 days group, A = significant vs. NC, B significant vs. Iso group, C = significant vs. ang1-7, D= significant vs. L-NAME,E =significant vs indomethacin,F= significant vs Both.

Table (2): Lipid profile in different studied groups at basal, 2 and 8 days

	Group Days	NC	ISO-MI	ISO+Ang-(1-7)	ISO+Ang-(1-7)+L-NAME	ISO+Ang-(1-7)+Indomethacin	ISO+Ang-(1-7)+both
Cholesterol (mg/dl)	Basal	88.05 ±4.49	84.52±3.36	85.82±4.07	86.15±3.43	82.75±4.31	82.42±2.46
	Day2	84.68 ±3.7	120.08±6.25 € A	105.43±2.7 € A	116.45±11.12 € A	108.41±3.88 € A	119.11±2.8 € A
	Day8	90.76 ±4.05	112.56±5.7	96.98±1.6 Ω B D E F	122.23±4.69 C	120.3±3.08 C	134.42±3.77 CDE
TG((mg/dl)	Basal	78.85 ±5.62	79.68±4.53	80.48±5.63	81.6±2.97	78.88±5.56	80.02±5.46
	Day2	79.53 ±4.83	113.23±3.5 € A	96.55±4.02 € A	104.01±3.87 € A	113.73±14.4 € A	115.2±2.7 € A
	Day8	78.32 ±1.27	94.71±0.39	82.81±0.48 Ω B D E F	110.45±5.1 C	109.28±3.51 C	128.57±3.66 CDE
LDL-CL(mg/dl)	Basal	23.53 ±7.11	20.1±3.35	21.05±4.29	21.77±4.29	18.23±5.41	17.85±4.73
	Day2	19.3± 4.56	69.08±7.45€ A	60.33±4.57€ A	65.47±11.2€ A	66.93 ±2.19€ A	70.7±3.28€ A
	Day8	26.42 ±5.08	55.56±4.67	31.27±5.43 Ω B D E F	59.92±5.59 C	61.4±2.69 C	78.67±3.97 C
HDL-CL(mg/dl)	Basal	48.55 ±2.69	48.63±1.47	48.68±1.42	48.12±2.44	48.75±1.64	48.6±2.14
	Day2	49.66 ±2.74	30.5±1.6	25.75±2.57€ A	30.08±1.08€ A	28.5±2.74€ A	27±2.73€ A
	Day8	48.83 ±1.66	32.13±1.74	41.18±1.63 Ω B D E F	30.17±1.53 C	31.28±2.56 C	30±1.87 C

The results were represented as mean ± SD, testes used are One way ANOVA and Turkey's post hoc (p≤ 0.05 is considered), NC = normal group, ISO=Isoprenaline, Both= L-NAME+ INDOMETHACIN, €=significant in comparison to basal group, Ω = significant significant in comparison to 2 days group, # = significant significant in comparison to 8 days group, A = significant vs. NC, B significant vs. Iso group, C = significant vs. ang1-7, D= significant vs. L-NAME,E =significant vs indomethacin,F= significant vs Both.

At day 8, serum Cholesterol, TG, LDL-CL were decreased significantly in Ang-(1-7) treated group when compared to ISO group as well as to L-NAME, indomethacin, combined (L-NAME& indomethacin) treated groups (p≤ 0.001) ,also HDL-CL was increased significantly in Ang-(1-7) treated group when compared to ISO group as well as to L-NAME, indomethacin, combined (L-NAME & indomethacin) treated groups (p≤ 0.001), Furthermore serum Cholesterol and TG

were increased significantly in combined group when compared to L-NAME, indomethacin groups.

Effect of Ang-(1-7), Ang-(1-7) +LNAME, Ang-(1-7) +Indomethacin Ang-(1-7) +both(L-NAME &Indomethacin) on ECG parameters(QRS,QT intervals, HR, and ST segment) :

ECG results are presented in table (3), Fig (1-3).There was no significant difference in PR interval duration among all groups. At day 0, lipid

profile results were not different between all groups. At day 2, the duration and voltage of QRS complex were decreased significantly while there was significant increase in QT interval duration, HR and S-T segment (p<0.001) in all studied groups in comparison with control group (p≤ 0.001). At day8, the duration and voltage of QRS complex were significantly increased while there was significant decrease in QT interval duration, HR and S-T segment (p<0.001) in Ang-(1-7) when compared to ISO group as well as to L-NAME, indomethacin, combined (L-NAME, indomethacin) treated groups (p≤ 0.001).

Table (3): ECG changes (PR, QRS, , QT intervals, HR, and ST segment) in different studied groups at basal, 2 and 8 days.

	Group Days	NC	ISO-MI	ISO+Ang-(1-7)	ISO+Ang-(1-7)+L-NAME	ISO+Ang-(1-7)+Indomethacin	ISO+Ang-(1-7)+both
PR interval(ms)	Basal	25.85± 1.84	24.92±1.04	26.63±2.13	26.83±4.32	25.2±1.66	25.1±1.75
	Day2	25.42± 1.78	26.02±1.83	26.53±1.84	26.95±1.94	25.1±2.09	24.8±1.9
	Day8	26.38± 1.45	25.77±1.56	25.38±1.52	26.53±1.56	25.62±1.66	26.75±1.64
QRS complex (ms)	Basal	45.5±1.87	45.42±3.49	47±2.36	46±3.16	46.5±2.07	45.05±3.13
	Day2	45.67± 2.07	37.2±1.04€ A	37.22±1.05€ A	37.2±1.04€ A	36.03±1.04€ A	37.07±.99€ A
	Day8	45.25± 2.72	38.33±0.51	45.34±0.76 Ω B D E F	39.13±1.55 C	39.83±1.83C	40±1.4 C
QRS complex (uv)	Basal	.35±.06	.39±.03	.44±.03	.39±.03	.37±.03	.36±.05
	Day2	.39±.03	.24±.008€ A	.25±.02€ A	.24±.02€ A	.27 ±.02€ A	.26±.03€ A
	Day8	.38±.03	.28±.03	.39±.02 Ω B D E F	.29±.05 C	.3±.02 C	.29±.04 C
QT interval(ms)	Basal	65.92± 4.41	67.23±2.33	66.83±1.59	64.12±1.52	64.95±.51	66.87±2.22
	Day2	67.23± 2.32	75.83±2.14€ A	80±6.32€ A	79.58±6.87€ A	79±3.74€ A	75.83±4.58€ A
	Day8	65.32± 2.64	75.17±4.02	69.33±.82 Ω B D E F	78.83±5.42 C	80.25±7.03 C	81±2.09 C
HR (bpm)	Basal	198.5± 7.8	204.5±4.7	198.8±3.97	202.16±7.9	195.83±8.14	200.5±6.77
	Day2	202.8± 6.11	313.8±20.8€ A	315±19.14€ A	302.7±19.76€ A	307.5±10.31€ A	305.5±17.21€ A
	Day8	199.5± 7.82	296.66±13.54	222.6±8.64 Ω B D E F	302.5±17.2 C	303.5±10.22 C	318±16.12 C
ST segment (mV)	Basal	.028±.009	.03±.008	.035±.015	.038±.016	.03±.008	.038±.015
	Day2	.03±.009	.12±.026€ A	.12±.021€ A	.11±.02€ A	.11±.02€ A	.11±.013€ A
	Day8	.03±.008	.12±.025	.07±.034 Ω B D E F	.13±.023 C	.13±.019 C	.13±.014 C

The results were represented as mean ± SD, testes used are One way ANOVA and Turkey's post hoc (p≤ 0.05 is considered), NC = normal group, ISO=Isoprenaline, Both= L-NAME+ INDOMETHACIN, €=significant in comparison to basal group, Ω = significant significant in comparison to 2 days group, # = significant significant in comparison to 8 days group, A = significant vs. NC, B significant vs. Iso group, C = significant vs. ang1-7, D= significant vs. L-NAME,E =significant vs indomethacin,F= significant vs Both.

Effect of Ang-(1-7), Ang-(1-7) +LNAME, Ang-(1-7) +Indomethacin Ang-(1-7) +both(L-NAME &Indomethacin) on myocardial morphology :

Pathological finding of different groups are represented in table (4). The histopathological changes score from all rats of the control group was A.-On the other hand, In SO- MI group, about 63% of rats became score C. The Ang-(1-7) treated group exhibited remarkable decrease in the damage score as about 63% of rats became score A.In Ang-(1-7) +L-NAME, Ang-(1-7) +Indomethacin Ang-(1-7) about 63%,75% of rats respectively became score C. In combined group about 87% of rats became score C. Figure (4. a) showed the normal architecture of the cardiac tissues. Figure (4.b) showed ISO- induced myocardium infarction characterized by extensive interstitial edema and neutrophil granulocytes in the infracted zone, with extensive myofibrillary degeneration. Treatment ISO-MI group with Ang-(1-7) resulted in slight degeneration of myocardium (Fig.4c). L-NAME and indomethacin produce edema of the interstitial tissue with infiltration of neutrophil and moderate muscle fiber degeneration(Fig.4d&e) when co-administrated with Ang1-7,on the other hand combined L-NAME and indomethacin(Fig.4f) resulted in sever edema of the interstitial tissue with marked infiltration of neutrophil indicating sever cardiomyocyte damage.-Noteworthy combined L-NAME and indomethacin administration produced more severe damage of myocardium than each one done separately.
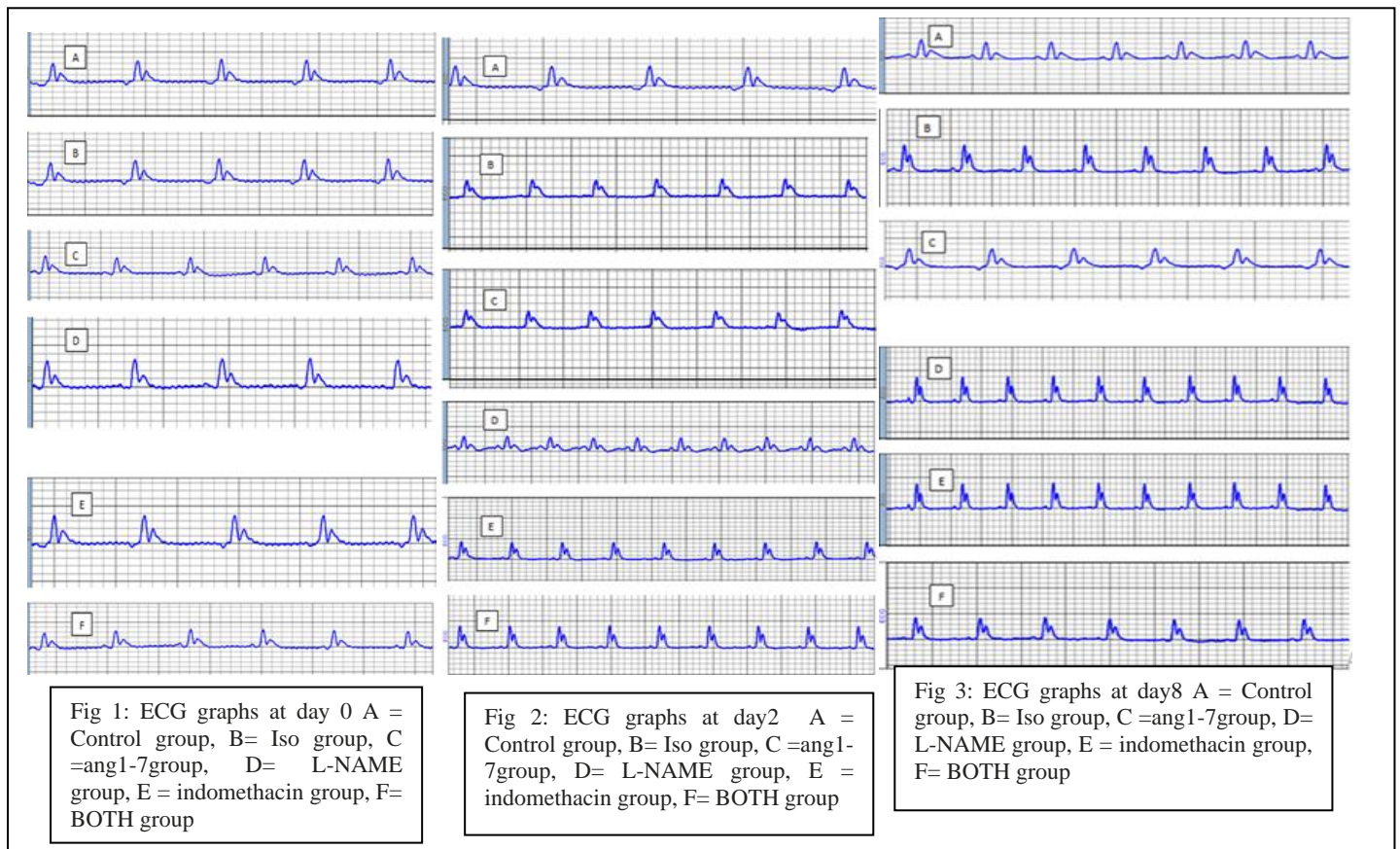

Fig 1: ECG graphs at day 0 A = Control group, B= Iso group, C =ang1-7group, D= L-NAME group, E = indomethacin group, F= BOTH group

Fig 2: ECG graphs at day2 A = Control group, B= Iso group, C =ang1-7group, D= L-NAME group, E = indomethacin group, F= BOTH group

Fig 3: ECG graphs at day8 A = Control group, B= Iso group, C =ang1-7group, D= L-NAME group, E = indomethacin group, F= BOTH group

Table (4): The myocardial histopathological damage score in different studied groups

	Group A	Group B	Group C
Normal Control	8 (100%)	-	-
ISO-MI	-	3(37%)	5(63%)
ISO+Ang-(1-7)	5(63 %)	3(37%)	-
ISO+Ang-(1-7) +L-NAME		3(37%)	5(63%)
ISO+Ang-(1-7) +indomethacin		2(25%)	6(75%)
ISO+Ang-(1-7)+both		1(12.5%)	7(87.5%)
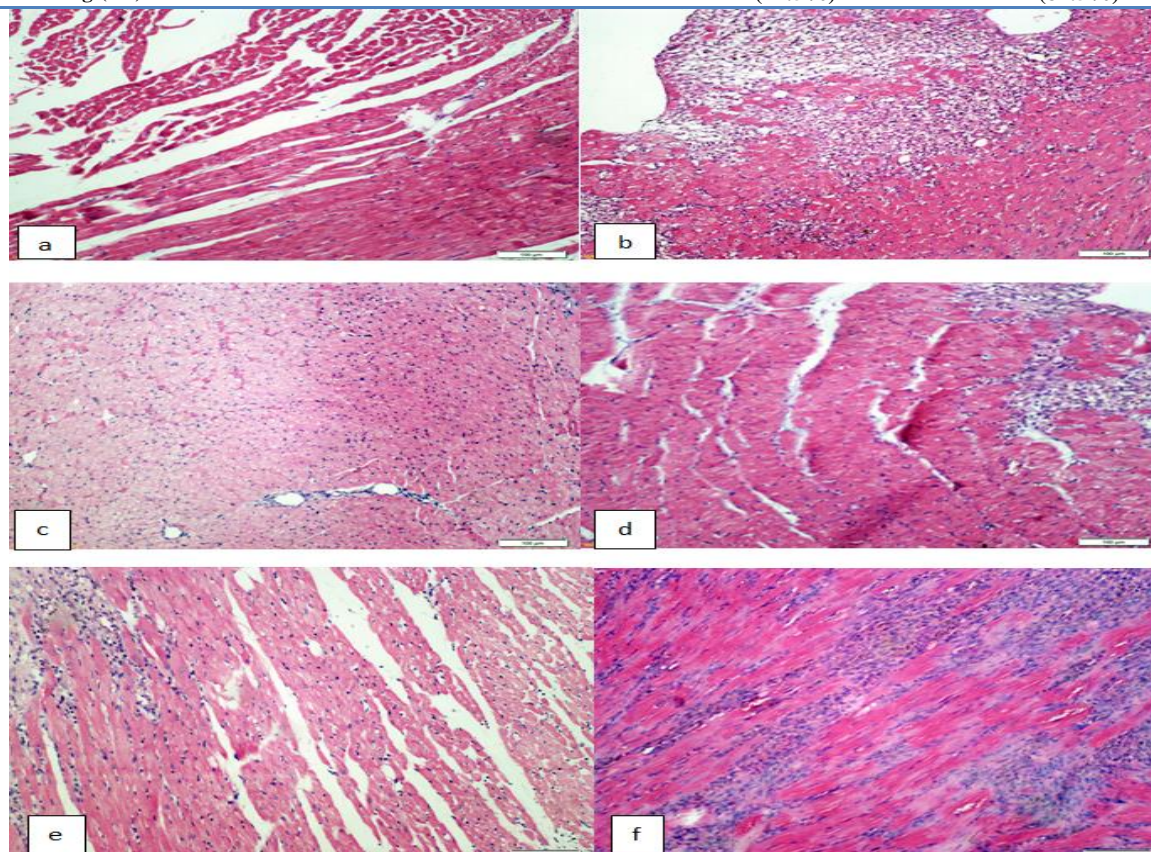
Figure 4: Specimens of the heart showing a) Normal structure of rat myocardium (control group), b) Infarcted zone with edema of the interstitial tissue with excess neutrophil indicating sever cardiomyocyte damage (ISO-MI group), c) zone of infarction with edema of the interstitial tissue ,mild infiltration of neutrophil and mild myofibrillary degeneration (ISO+Ang-(1-7)), d) edema of the interstitial tissue with moderate infiltration of neutrophil and moderatecardiomyoctes damage (ISO+ Ang-(1-7) +L-NAME group), e) edema of the interstitial tissue with moderate infiltration of neutrophil and moderatecardiomyoctes damage (ISO+ Ang-(1-7) +indomethacin group) and f) edema of the interstitial tissue with excessive infiltration of neutrophil and excessive cardiomyoctes damage (ISO+both group) (H&E, 200X).

Effect of Ang-(1-7), Ang-(1-7) +LNAME, Ang-(1-7) +Indomethacin Ang-(1-7) +both(L-NAME &Indomethacin) on immunohistochemistry of myocardial caspase 3 expression :

Caspase 3 results are presented in table (5), and fig (5a-f). Caspase 3 was increased significantly in ISO-MI group in comparison with control group.

Caspase 3 was decreased significantly in Ang1-7 treated group when compared to ISO-MI group as well as to L-NAME, indomethacin, combined (L-NAME& indomethacin) treated groups (p≤ 0.001). Caspase 3 increased significantly in combined treated group when compared to L-NAME, indomethacin groups (p≤ 0.001).

Table (5): Comparison of caspase 3(integrated density$*10^6$) among different groups

		Caspase 3
NC	Mean	.16±.007
ISO-MI	Mean	49.1±5.7 A
ISO+ANG1-7	Mean	7.5±1.36 a b d e f
ISO+ANG1-7+L-NAME	Mean	28.9±3.3 a c
ISO+ANG1-7+INDOMETHACIN	Mean	24.9 ±2.9a c
ISO+ANG1-7+BOTH	Mean	54.1± 2.8 c d e

The results were represented as mean ± SD, testes used are One way ANOVA and Turkey's post hoc (p≤ 0.05 is considered), NC = normal group, ISO=Isoprenaline, Both= L-NAME+ INDOMETHACIN, a = significant vs. NC, b significant vs. Iso group, c = significant vs. ang1-7, d= significant vs. L-NAME,e =significant vs indomethacin,f= significant vs BOTH.
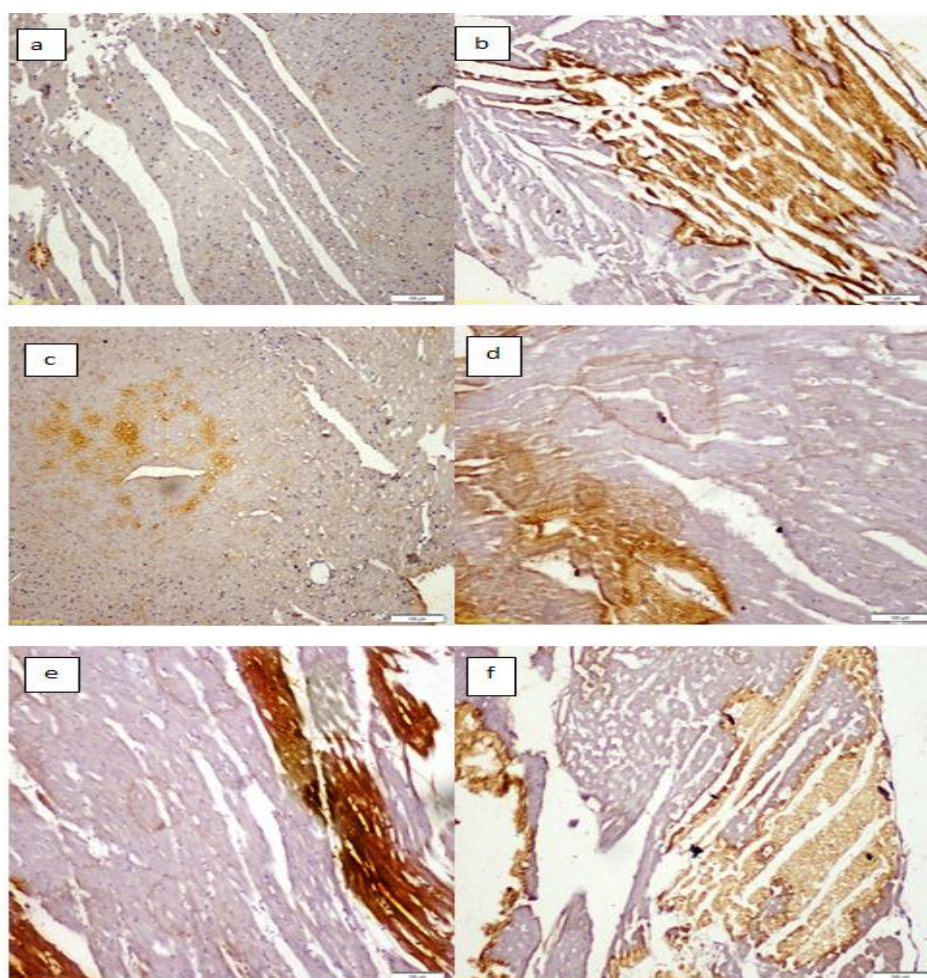
Figure (5): Immunohistochemical stained myocardial tissue sections for Caspase3 a)normal control group showing minimal amount of caspase 3,b)ISO group showing large amount of caspase 3,c) Ang-(1-7)treated group showing little amount of caspase 3,d) Ang-(1-7)+LNAME treated group showing large amount of caspase 3, e) Ang-(1-7)+Indomethacin treated group showing large amount of caspase 3, f) from Ang-(1-7)+L-NAME+ Indomethacin treated group showing more large amount of caspase 3. Caspase 3 is shown in figures (light brown color).

Discussion

This work was designed to study the possible cardioprotective effect of Ang-(1-7) in rat model of MI, and the prospective role of NO and PGs in this probable effect of Ang1-7. These effects were investigated on a documented rat model of ISO-induced myocardial infarction through evaluation of cardiac enzyme, lipid profile, ECG changes, caspase 3 expression, cardiac tissue remodeling(تحذف) and histopathological changes.

The results of the study showed that high dose of isoprenaline (150 mg/Kg) induce cardiac damage similar to what occurs in acute infarction. This is in agreement with Siddiqui et al (25) and Asdaq et al (26).

The results of the current study showed high serum levels of cardiac enzymes in ISO-treated groups indicating cardiomyocytes damage. These results were in line with studies before (27,28,29).These results could be explained by cytoskeleton disorganization (30), cellular damage and loss of functional integrity and permeability of cell membrane (31).

Ang-(1-7) treated group showed decreased serum levels of cardiac enzymes denoting cardioprotective effect of this heptapeptide. These finding were in agreement with other studies (30, 32, 33).The decrease in these cytosolic enzymes may be explained by Ang-(1-7) ability to reduce oxidative stress, dysfunction of mitochondria, and mitochondria-dependent apoptosis (32), thus decreasing the size of infarcted area and reducing the leakage of enzymes from cardiomyocytes (4).

However, the decrease in cardiac enzymes was not observed when Ang - (1–7) administered along with L-NAME and /or indomethacin indicating that NO and PGs prevent cardiomyocyte damage and significantly reduced the size of infarcted area, these finding were in agreement with Burley et al., (13) and Xiao et al.,(34).These findings indicate the cardioprotective effect of Ang - (1–7) may involve NO and/or PGs which are in accordance with Dias-Peixoto et al (35) and Ferreira et al,(36).Noteworthy combined L-NAME and indomethacin produce more deleterious effect than each done separately indicating additive effect of NO and PGs.

It has been demonstrated that the mechanism through which NO can preserve cardiomyocyte integrity involve vasodilation of coronary vessels (23), in addition to activation of the PI3K/Akt pathway-which is an important cell-surviving pathway-(35).PGs hinder cardiomyocyte injury through increase the level of cAMP inducing vasodilation of coronaries (37). Also, PGs increased [cAMP] in cardiomyocytes thus preventing secretion of TNF-α which play a role in aggravating cardiac injury (34).

There was disturbed lipid profile (high serum cholesterol, TG, LDL-CL, and decreased HDL-CL) in ISO-treated groups denoting myocardial injury. These findings were in accordance with Shirafkan et al. (38) and Al-Yaha et al,(39). The mechanism of disturbed lipid profile after MI could be explained by elevated flux of fatty acids and impaired removal of VLDL from the plasma (40), excessive production of LDL-CL (38), increasing the activity of endothelial lipase and soluble phospholipase A2 (41).

The data of the present study demonstrated improvement of lipid profile in Ang-(1-7) treated group in the form of increased level of HDL-CL with decreased level of total cholesterol, TGs, and LDL-CL suggesting potent antioxidant property of Ang-(1-7) , which guard against oxidative damage of cardiomyocytes and preserve integrity of cell membrane thereby improving cell viability. These finding were in harmony with Zhao et al.,(42), Santos et al., (9) and Santos et al.,(43).

It was proved that the mechanisms account for regulation of lipid metabolism by Ang-(1-7) include the vasodilation which stimulates the clearance of HDL particles by the liver (9), and increases the cholesterol transport. Also, Ang-(1-7) increases the expression adipose lipid-binding protein that is involved in esterification of fatty acids (43).

Co-infusion of L-NAME and or indomethacin with Ang-(1-7) prevent the effect of Ang-(1-7) on lipid profile indicating that Ang-(1-7) action is mediated through NO or PGs or both (44,45,46).

The biochemical changes of the current work were supported by ECG finding.

ISO-treated groups showed disturbed ECG parameters in the form of increased heart rate, shortened durations of QRS complex, R-R intervals, and prolongation of QT interval with no change in PR interval. There was also significant elevation in the ST segment and decreasing QRS complex amplitude. These changes of ECG segments are a definite criterion for diagnosis of MI-(47), indicating ischemic necrosis, disturbance of myocyte membrane integrity(48), and generation of oxidative stress, highly toxic oxygen derived free radicals (27) that produces many physiological and functional changes in heart (49).

The QT interval prolongation is caused by cardiac vagal dysfunction and indicates cardiac toxic potential (49).ST-segment elevation indicating consecutive loss of cellular membrane fluidity and permeability (50).

Increased heart rate and reduced RR interval reflect myocardial edema, it causes dysfunction of myocardium (51).It may occur as a sequence of decreased mitochondrial

energetic as well as impaired Ca^{+2} transport leading to intracellular Ca^{+2} overload (52).

Noteworthy, we observed that ang1-7 treated group showed cardioprotective effect of this heptapeptide evidenced by decreased the effect of ISO on heart rate , in addition to significant restoration of QRS complex (duration and voltage), R-R interval and QT interval duration, beside decreased ST segment elevation as compared to ISO group. Restoring these parameters indicates that Ang-(1-7) has a role in maintaining integrity of cellular membrane; because of its ability to minimize the decrease in action potential duration, as well as suppressing the expression of Ca^{+2} channel and outward K^+ channel (53).Thus Ang-(1-7) has the ability to change ion channel and calcium handling protein expression such as SERCA and ryanodine receptors (54). In addition Ang-(1-7) activates the Na^+-k^+ pump, hyperpolarizes the cardiomyocytes and modulate the propagation of impulse (55), resulting in modulation of cardiac function such as excitability and contractility through affecting excitation-contraction coupling (56).

On the other hand co-infusion of L-NAME and or indomethacin with Ang-(1-7) reverse this cardioprotective effect of Ang-(1-7) indicating that Ang-(1-7) action is mediated through NO (57,58) or PGs (59) or both (7).

The cardioprotective effect of NO could be explained by the fact that NO increased the level of cGMP ,regulating Ca^{2+} homeostasis thus affecting the genesis of the cardiac action potentials and guard against cardiac arrhythmias(60),such effect is supported by ability of NO to inhibit of responsiveness to sympathetic stimulation (61).Also, PGs could activate ATP-sensitive k^+ channels In the heart resulting in decreased intracellular Ca^{+2} , prevention of mitochondrial Ca^{+2} overload and preservation of the myocardial energy status (62).

ECG changes were confirmed by pronounced pathological changes in the myocardium. Lobo Filho et al., (21) and Li et al., (63) reported that ISO-induced marked necrosis, fibrosis, and degeneration of cardiomyocytes with neutrophil infiltration and edema of interstitial tissue. In agreement with these studies, results of the current work demonstrated these finding in ISO treated group, while Ang-(1-7) treated group exhibited reduction in the damaged area caused by ISO administration. These finding were in agreement with Marques et al., (3) and Gomes et al., (64).

These results could be explained by the anti-inflammatory properties of Ang-(1-7)(65).In addition, Ang-(1-7) increases VEGF (a marker of angiogenic cells), and stimulates bone marrow–derived cardiovascular progenitor cells suggesting that Ang-(1-7) stimulates the generation of new capillaries and promotes cardiac regeneration after MI (66),thereby

reducing the infarcted size (67) .On the other hand; administration of L-NAME and or indomethacin along with Ang-(1-7) prevent this cardioprotective effect of Ang-(1-7) indicating that Ang-(1-7) action is mediated through NO (68) or PGs (45) or both (69).

These data can be explained by anti-inflammatory effect of NO via inhibition of proinflammatory cytokines (70), beside its role in prevention of oxidative stress damage (71), PGs has an inhibitory effect on the neutrophil function, in addition to regulation of the release of cytokines in macrophages, Also, PGs could stimulate the production of hepatocyte growth factor in non-cardiomyocytes which has a growth-promoting effect on various types of cells (34).

As regard to Caspase-3 expression, the current study revealed that the expression of Caspase-3 protein was increased in ISO-MI group. This result is in line with Li et al., (63) and Zidar et al., (72).

The stimulation of the apoptotic cascade leads to cleavage of caspase -3. The peptide end of active caspase -3 represents a new epitope that is not present in normal cells. So, the detection of this novel epitope has been proved to be a unique, specific and sensitive indicator of apoptosis (72).

The data of the present study clearly demonstrated that Ang-(1-7) decreases caspase 3 activity in infarcted rats that is in line with Meneses et al., (73) and Liu et al., (74) denoting the remarkable role of Ang-(1-7) in decreasing the size of infarcted area and prevention of apoptosis .These observations can be clarified by ability of Ang-(1-7) to reduce mitochondrial dysfunction, oxidative stress, and mitochondria-dependent apoptosis (32).

However, the decrease in caspase -3 activity was not observed when Ang - (1–7) administered along with L-NAME and /or indomethacin. This observations suggests that the cardioprotective effect of Ang - (1–7) may involve NO and/or PGs (36, 69).

These finding could be explained by the antiapapoptotic effect of the NO/cGMP/PKG pathway that results in regulation of Ca^{+2} homeostasis, in addition to inhibition of caspases3,9 by S-nitrosylation of the catalytic-site these of caspases (42).Beside, PGs act through prevention of mitochondrial Ca^{+2} overload , preservation of the myocardial energy status ,and prevention of oxidative stress induced damage(62).

In conclusions Ang-(1-7) is one of the cardioprotective components of RAS. This action of Ang-(1-7) is mediated through NO and PGs where they have synergistic effect. NO and PGs mediated Cardioprotective effect of Ang-(1-7)thought to be due to vasodilation of coronaries, anti-inflammatory, antioxidant effect, preserving cell permeability and gap

junction function, decreasing caspase 3 expression in the myocardium .

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Abbreviation

Akt	**Protein Kinase B**
L-NAME	**N(ω)-nitro-L-arginine methyl ester**
MAP kinase	**Mitogen-activated protein kinase**
MAS	**Ang- (1-7) receptor, G protein–coupled receptor**
SERCA	sarco/endoplasmic reticulum Ca^{2+}-ATPase