Effect of Rosuvastatin and Ramipril on Angiogenesis in Drug-Induced Myocardial Infarction in the Adult Albino Rat

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Abstract: Introduction: Cardiovascular disease is considered the major cause of morbidity and mortality throughout the world. Epinephrine is a natural catecholamine that simulates the actions of sympathetic nervous system (SNS) on the heart. Epinephrine administration in large doses causes changes similar in direction and magnitude to those found in the heart following myocardial infarction (MI). Angiogenesis takes place as a compensatory mechanism to provide blood supply to the ischemic myocardium. Enhancing angiogenesis -by using drugs- causes amelioration of MI and preservation of myocardial function. With the use of animal models in preclinical research, workers have acquired a large amount of information on the pathogenesis/progression of MI, which has aided the development of effective treatment options. Rosuvastatin and ramipril are drugs usually given in patients with MI as first line therapy. **Aim of the Work:** to explore the angiogenic effect of rosuvastatin in an experimentally-induced acute myocardial infarction in the adult albino rats. This study also aimed to reveal if ramipril has a stimulatory or inhibitory effect on angiogenesis or not. **Materials and Methods:** Fifty adult Wistar albino rats were maintained for 34 days as follows: Group 1, control group, group 2, epinephrine group [induction of MI by 2mg/kg/day, subcutaneously for 2 days], Group 3, ramipril group [induction of MI as group 2, then, administration of 3mg/kg/day ramipril, via gastric gavage for 30 days], group 4, rosuvastatin group [induction of MI as group 2, then, administration of 10mg/kg/day rosuvastatin, via gastric gavage for 30 days], group 4, rosuvastatin + ramipril group [induction of MI as group 2, then, administration of both 10mg/kg/day rosuvastatin and 3mg/kg/day ramipril, via gastric gavage for 30 days]. At the end of experimental study, cardiac tissues were taken for histological studies. **Results:** Comparing with the control it was found that, administration of epinephrine showed marked myocardial infarction and fibrosis. Administration of rosuvastatin alone or ramipril alone caused reduction of MI and fibrosis, but rosuvastatin group only showed marked angiogenesis. Simultaneous administration of rosuvastatin and ramipril achieved the best results according to reducing MI and fibrosis, but achieved the same picture of angiogenesis in rosuvastatin group. **Conclusion:** This study revealed that, epinephrine in large doses can produce MI and fibrosis. These lesions were greatly ameliorated by administration of both rosuvastatin and ramipril, with marked angiogenesis in the infarcted areas.

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1. Introduction

Cardiovascular diseases account for a high rate of deaths all over the world. According to World Health Organization (WHO), cardiac disorders are the second leading cause of death with an estimate of 7.8 million deaths in 2013 and it has been predicted that the number of deaths will greatly increase to 9 million by the year 2025 **[Baldwin et al., 2013]**. Myocardial infarction (MI) forms a major category among the cardiovascular disease***[Ferraro et al., 2013]***.

Myocardial infarction (MI) is the rapid development of myocardial necrosis caused by imbalance between myocardial oxygen supply and demand, resulting in myocardial hypoxia which ends eventually by myocardial fibrosis ***[*Rajadurai *and Prince, 2007]***.

The metabolic disarrangements that occur during myocardial ischemia predisposes for the formation of free radicals named reactive oxygen species (ROS) ***[Bandyopadhyay et al., 2004]***. Oxidative stress resulting from increased production of free radicals is related to decreased levels of antioxidants in the myocardium ***[Das and Maulik, 2000]***. Body compensatory mechanisms occur during MI releasing some important factors as a trial to maintain blood supply to the ischemic area and also reduce the size of the infarcted area **[Padmanabhan and Prince, 2006]**.

So, better understanding of the processes involved in the pathophysiology of myocardial infarction has led to the search for drugs which decrease the processes aggravating MI and enhancing body compensatory mechanisms minimizing the effect

of MI **[Skyschally et al., 2008]**.

Angiogenesis is the process of formation of new blood vessels from the existing ones in response to ischemia or other kinds of stress **[Sata et al., 2004*]**. This is beneficial in some ischemic conditions **[Jujo et al., 2008*]**. Angiogenesis takes place as a compensatory mechanism to provide blood supply to ischemic tissues and preserves blood flow in ischemic organs, such as heart or limb **[Serini et al., 2008]**.

Therapeutic angiogenesis is a new strategy that has been emerged to treat tissue ischemia by promoting the proliferation of the collateral vessels ***[Renault and Losordo, 2007]***. This strategy received a great attention and emerged as one of the most exciting and promising therapies in ischemia such as myocardial infarction ***[Kwon et al., 2008]***.

In myocardial infarction, the incidence of hypoxia-induced angiogenesis is suggested to be due to an increase in the expression of some important growth factors, such as vascular endothelial growth factor (VEGF) ***[Hassab El Nabi and Kamha, 2012]***. VEGF expression is regulated by hypoxia, oxidative stress and nitric oxide (NO) **[Siddiqui et al., 2004]**. NO has been reported to be an important mediator in the cardiovascular system and play an important role in reducing the infarct size during myocardial infarction ***[Shah and MacCarthy, 2000]***.

It has been demonstrated that NO and VEGF are over expressed and promote tumor angiogenesis and metastasis. In addition, NO promotes the survival of ischemic tissue by stimulating angiogenesis ***[Zhou et al., 2013]***.

Rosuvastatin is a new highly effective member of statins. Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, the rate-limiting enzyme in cholesterol biosynthesis. This category was used mainly in treatment of dyslipidemia ***[Ong, 2005]***.

Some clinical trials established the benefits of statins (especially rosuvastatin) on the coronary heart diseases, ischemic cerebrovascular events (such as stroke) and limb ischemia ***[Sata et al., 2004]***.

Statins were reported to improve the endothelial function by mechanisms that are unrelated to the cholesterol lowering effect. Some of these mechanisms involve the prevention of inflammation, down regulation of the systemic inflammatory response and induction of angiogenesis ***[Stojadinovic et al., 2010]***.

The renin–angiotensin system (RAS) is an important factor with implication in the pathogenesis of myocardial ischemia reperfusion injury. As a result, pharmacologic blockade of this system by angiotensin converting enzyme inhibitor (ACE-I) has been an attractive strategy for cardioprotection against ischemia reperfusion injury ***[McMurray, 2008]***.

A renin-angiotensin system (RAS) is present in the heart. Its activation in myocardial ischemia, leads to an increased formation of local Angiotensin II (Ang II) ***[Frohlich and Re, 2009; Safari et al., 2012]***.

Ang II has been shown to exert a direct vasoconstrictor effects on the coronary arteries. It is also regarded as a pro-oxidant because it can stimulate the production of reactive oxygen species (ROS) ***[Dandona et al., 2003]***. Therefore, blockade of renin angiotensin system (RAS) by an angiotensin converting enzyme inhibitor (ACE-I) could reduce oxidative stress and could be beneficial for prevention of MI ***[Wu et al., 2009]***.

Angiotensin II has an angiogenetic effect. ACE inhibitors may not totally block the vascular formation of Ang II ***[Frohlich and Re, 2009]***. So, ACE inhibitors block the vasoconstrictor effect of Angiotensin II but may interfere with its angiogenetic effect ***[Safari et al., 2012]***.

Ramipril is a member of angiotensin converting enzyme inhibitor (ACE-I). This category was used mainly in the past for treatment of hypertension, but nowadays it is widely used in cardiovascular diseases (especially myocardial ischemia) even in non-hypertensive patients ***[Woo and Probstfield, 2009]***.

2. Material and Methods

Animals Used:

Fifty adult male and female Wistar albino rats aging 12-14 weeks and weighing 250-300 gm were used in this study. The animals were obtained from the animal house, Faculty of Medicine, Cairo University. They were housed in a wire mesh cages in a well-ventilated room, and were kept under a constant day/night cycle in a climate controlled condition with free access to food and water. All experiments were carried out according to the guidelines of the Institutional Animal Ethics Committee.

Chemicals and drugs:

The chemicals used in this work were:

- Epinephrine: in the form of Epinephrine 1mg ampoules, produced by Misr Pharmaceutical Company, Kasr El-Nile Quarter, Cairo, Egypt.
- Rosuvastatin: in the form of Crestor 5mg tablets, produced by AstraZeneca Egypt, Sixth of October City, Giza, Egypt; under the license of AstraZeneca Multinational Pharmaceutical Company, Cambridge, United Kingdom.
- Ramipril: in the form of Tritace 5mg tablets, produced by Sanofi Egypt (S.A.E), El-Zeitoun, Cairo, Egypt; under the license of Sanofi-Aventis, Germany.

Experimental Design:

After two weeks of acclimatization, the rats were randomly divided into five groups; ten rats in each group (five males and five females) as follows**:**

Group I (control group):

Rats were injected subcutaneously by 1 ml distilled water once daily for two consecutive days (24 hours apart) adopted by ***[Huda and Akhter, 2014; Parvin and Akhter, 2008].***

Group II (epinephrine treated group):

Rats were given epinephrine (2 mg/kg) once daily by subcutaneous injection for two days (24 hours apart) to induce an acute myocardial infarction adopted by ***[Huda and Akhter, 2014; Parvin and Akhter, 2008].***

Group III (ramipril treated group):

Rats were treated in the same manner as group II and then were given ramipril (3 mg/kg/day) -after dissolution of the tablet in 5ml water- by gastric gavage started two days after induction of infarction "from the last injection" and continued for 30 days adopted by ***[Bayir et al., 2012; Keles et al., 2009].***

Group IV (rosuvastatin treated group):

Rats were treated in the same manner as group II and then were given rosuvastatin (10 mg/kg/day) -after dissolution of the tablet in 5ml water- by gastric gavage started two days after induction of infarction "from the last injection" and continued for 30 days adopted by ***[Huang et al., 2015].***

Group V (ramipril- and rosuvastatin-treated group):

Rats were treated in the same manner as group II and then were given both ramipril (3 mg/kg/day) and rosuvastatin (10 mg/kg/day) simultaneously by gastric gavage started two days after induction of infarction "from the last injection" and continued for 30 days.

Specimens collection:

The rats in all groups were sacrificed by the end of the 34th day, except the rats in the epinephrine-treated group; three rats were sacrificed by the end of the 4th day while the rest of rats in this group were left for observation. At the end of each determined period, the rats were anaesthetized using ether inhalation. Then, the heart of each rat was dissected out, preserved in 10% buffered formalin and then processed for paraffin sections to be examined under the light microscope.

Processing of the specimens for light microscopic examination:

The specimens were processed for paraffin sections by gradual dehydration using ascending graded concentrations of alcohol, cleared in xylene and embedded in soft and then in hard paraffin wax.

Sections of 5 microns thick were cut by microtome and were subjected to the following stains:

1. Hematoxylin and Eosin (H & E): for evaluation of histopathological changes.
2. Masson's trichrome stain: mainly for detection of the collagenous structures in the heart; like collagen in between cardiomyocytes and also, collagen in and around the wall of blood vessels.

3. Results

Generally, throughout the study, the rats received epinephrine in all groups suffered from an increased body weight, anorexia, diminished activity and became lethargic. These observations were diminished in ramipril-treated rats; more diminished in rosuvastatin-treated rats; and -nearly- completely diminished in rats treated with combined rosuvastatin and ramipril to achieve normal growth and activity by the end of the 3rd week, like the control group.

The death rate, in our study, was very high among the treated groups. The control group showed no death; while the epinephrine-treated group had the highest death rate as 4 male rats died before the 4th day of the study (i.e. before sacrification), 3 rats were sacrificed, while the rest of rats were left for observation and died by the end of the 6th day. In ramipril-treated group, 7 rats (5 male and 2 female) died by the end of the 9th day; while in rosuvastatin-treated group, 6 rats (5 male and 1 female) died by the end of the 10th day. The combined rosuvastatin- and ramipril-treated group showed the least death rate among rats as only 3 male rats died by the end of the 10th day. Moreover, no deaths occurred after the 10th day of the study.

There was no difference concerning the external appearance of the hearts excised from all groups.

Histological Results:

A. Hematoxylin and Eosin stained sections:

The cardiac tissue of the adult albino rat of the control group showed viable myocardium, with an intact and well distinct cell borders, average cytoplasm, oval centrally located one nucleus or two nuclei. Some nuclei had one nucleolus, while the others had two prominent nucleoli. Moreover, there were some scattered blood vessels in the myocardium (Figs. 1A,2A & 3A). The myocardial fibers appeared interlacing with branching of cardiomyocytes at acute angles. Therefore, they appeared longitudinally and transversely arranged in the same section. Furthermore, there were apparent cross striations (Figs. 2A & 3A).

The cardiac tissue of the adult albino rat of the epinephrine-treated group showed multiple areas of myocardial infarctions in the form of sub-endocardial, intra-myocardial and sub-pericardial infarctions. The sub-endocardial and the sub-pericardial infarctions had the form of contraction band necrosis (necrosis of group of adjacent cardiomyocytes); while the intra-myocardial infarction had the form of focal necrosis of cardiomyocytes (Fig. 1B). Moreover, there were variable areas of myocardial hemorrhage appeared within the infarcted myocardium (Figs. 1B & 2C). In the infarcted areas, myocardial cells appeared totally necrotic with lost cell borders, pale eosinophilic cytoplasm, and scattered mononuclear inflammatory

cells. The nuclei of some cells appeared pyknotic with no nuclei in the other cells (Figs. 2B & 3B). Furthermore, some sections in this group showed completely occluded blood vessels by thrombus attached to its wall (Fig. 3C).

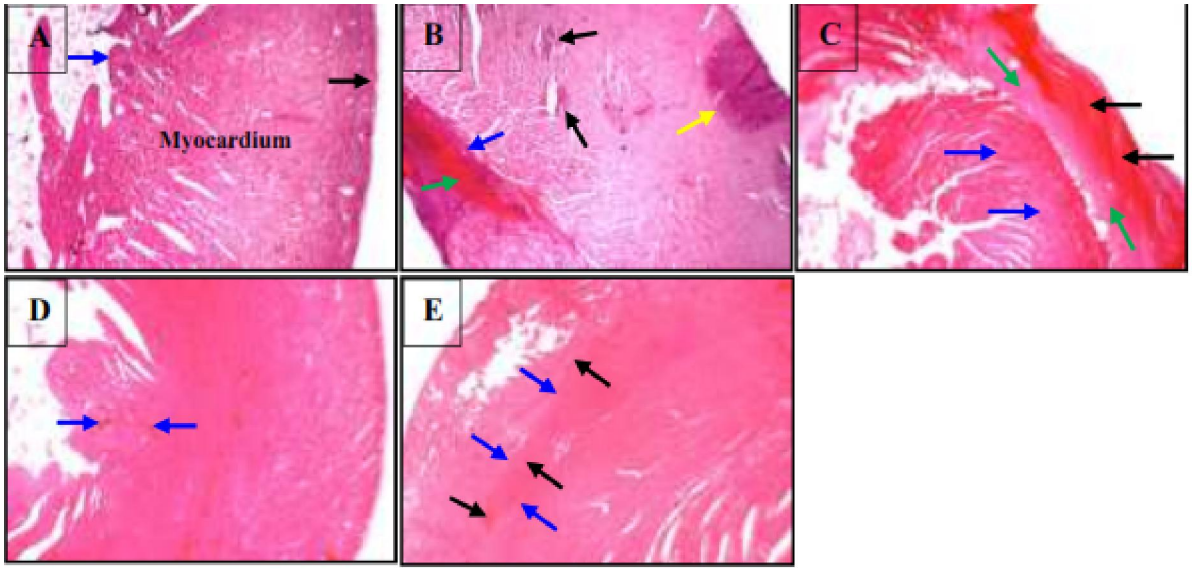

Fig. 1: Photomicrographic sections from the heart of adult albino rats (H & E, X 100). **A-** Control group showing viable myocardial tissue with an intact pericardium (black arrow) and an intact endocardium (blue arrow). **B-** Epinephrine-treated group showing multiple infarct areas in the form of sub-endocardial (blue arrow), intra-myocardial (black arrows) and sub-pericardial (yellow arrow). There is also myocardial hemorrhage within the infarcted myocardium (green arrow). **C-** Ramipril-treated group showing an area of infarction (blue arrows), covered by thick area of fibrinous layer (black arrows), with an area of edema in between the previous areas (green arrows). **D-**Rosuvastatin-treated group showing many angiogenic blood vessels with irregular outlines (blue arrows), with partially viable myocardial tissue and eosinophilic cytoplasm. **E-** Combined rosuvastatin- and ramipril-treated group showing homogenous pink area of myocardium denoting recovery of inflammation (blue arrows) with scattered angiogenic blood vessels within and surrounding this area (black arrows).

The cardiac tissue of the adult albino rat of the ramipril-treated group showed the three stages of inflammation which occurs in MI; an area of infarction (healing stage), covered by thick fibrinous layer (stage of active inflammation), with intervening area of edema in between the previous areas (late stage of inflammation) (Figs. 1C & 2D). In the infarcted area, the sections showed partially viable myocardial tissue, with partially preserved cell borders, eosinophilic cytoplasm and some scattered mononuclear inflammatory cells. The nuclei of most cells appeared pyknotic, some cells appeared with normal nuclei, while others had no nuclei (Fig. 3D).

The cardiac tissue of the adult albino rat of the rosuvastatin-treated group showed many new blood vessels with irregular outlines which denotes angiogenesis (Figs. 1D & 2E). The sections also showed partially viable myocardial tissue with partially preserved cell borders, some normal nuclei and other pyknotic nuclei, some cardiac muscle fibers had no nuclei, and some scattered mononuclear infiltrate (Fig. 3E).

The cardiac tissue of the adult albino rat of combined ramipril- and rosuvastatin-treated group showed homogenous pink area of myocardium denoting MI and recovery of inflammation with scattered blood vessels within and around this area (Fig. 1E). The sections also showed more viable myocardial tissue with more viable normal nuclei and scattered mononuclear inflammatory infiltrate. Moreover, proliferating blood vessels with irregular outlines were visualized in comparison with the normal blood vessels which had regular outlines in the same section (Figs. 2F & 3F).

B. Masson's trichrome stained sections:

Histological examination of sections of the adult albino rats of the control group showed the normal collagen distribution in the heart. There was a rounded thick-walled blood vessels with a collagen layer around it. There was also a very thin collagen layer -or nearly absent- in between the muscle fibers (Fig. 4A).

Histological examination of sections of the adult albino rats of the epinephrine-treated group

showedan area of myocardial necrosis with severely distorted cardiomyocytes and lost cell borders with no obvious fibrosis denoting active stage of MI (Fig. 4B). Another sections showed an area of healed MI with indistinct cell borders and thick irregular collagen bundles in between cardiac muscle fibers (Fig. 4C).

Histological examination of sections of the adult albino rats of the ramipril-treated group showed: partially preserved cell borders with thin irregular collagen fibers in between cardiac muscle fibers and also, around thrombosed thick-walled blood vessel (Fig. 4D).

Histological examination of sections of the adult albino rats of the rosuvastatin-treated group showedpartially viable myocardial tissue with partially preserved cell borders. There were clearly visualized blood vessels with irregular outlines and RBCs in their lumen surrounded by thin irregular collagen layer. Also, there were mildly thick collagen fibers in between cardiac muscle fibers (Fig. 4E).

Histological examination of sections of the adult albino rats of combined ramipril- and rosuvastatin-treated group showed: more viable myocardial tissue with preserved cell borders, thin irregular collagen layer around proliferating thin-walled blood vessels and in between cardiac muscle fibers. Other cells showed no collagen fibers in between them like the normal control group (Fig. 4F).

4. Discussion

Myocardial infarction (MI) is one of the most severe health problems in terms of both mortality and morbidity. The most important difference between MI and these other diseases is the sudden onset of MI; the vast majority of deaths happen in the first few hours of its incidence ***[Suthahar et al., 2017]***. If an individual lives beyond the acute phase of MI, many complications associated with MI ensue. Among these complications, perhaps the most important is ventricular failure due to fibrosis, which clinically emerges as varying degrees of heart failure ***[Muthuramu et al., 2014]***.

Therefore, many studies have been conducted and many medications were found to prevent MI and decrease post-MI complications. Among these medications were rosuvastatin and ramipril ***[Huda and Akhter, 2014; Muse et al., 2017; Oesterle et al., 2017]***.

The present work aimed mainly to study the effect of rosuvastatin and/or ramipril on angiogenesis after induction of myocardial infarction (MI) by epinephrine in an adult rat model.

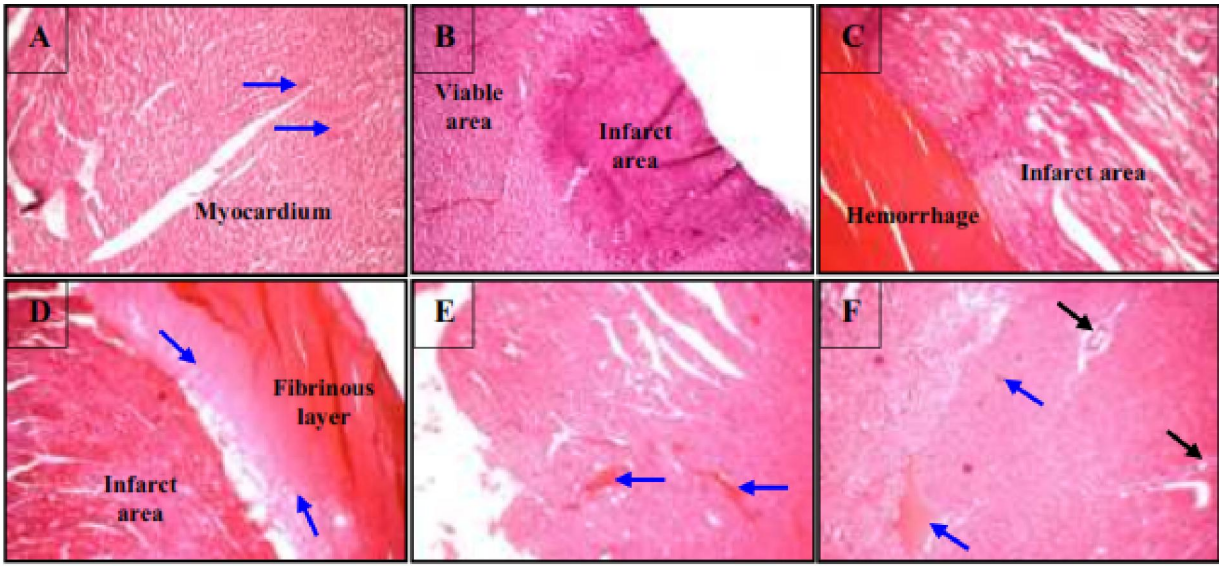

Fig. 2: Photomicrographic sections from the heart of adult albino rats (H & E, X 200). **A-** Control group showing viable myocardial tissue with scattered blood vessels (blue arrows). **B-**Epinephrine-treated group showing sub-pericardial infarction with lost cell borders compared to viable myocardium. **C-**Epinephrine-treated group showing sub-endocardial infarction with lost cell borders and pale eosinophilic cytoplasm. There is also an area of myocardial hemorrhage within the infarcted myocardium appears as organized hematoma. **D-** Ramipril-treated group showing the three stages of inflammation; an area of infarction, covered by thick fibrinous layer, with intervening area of edema in between the previous areas (blue arrows). **E-** Rosuvastatin-treated group showing many new blood vessels with irregular outlines (blue arrows), with partially viable myocardial tissue, and eosinophilic cytoplasm. **F-** Combined rosuvastatin- and ramipril-treated group showing more partially viable myocardial tissue and proliferating thin-walled angiogenic blood vessels with irregular outlines (blue arrows), compared to normal thick-walled blood vessels with regular outlines (black arrows).

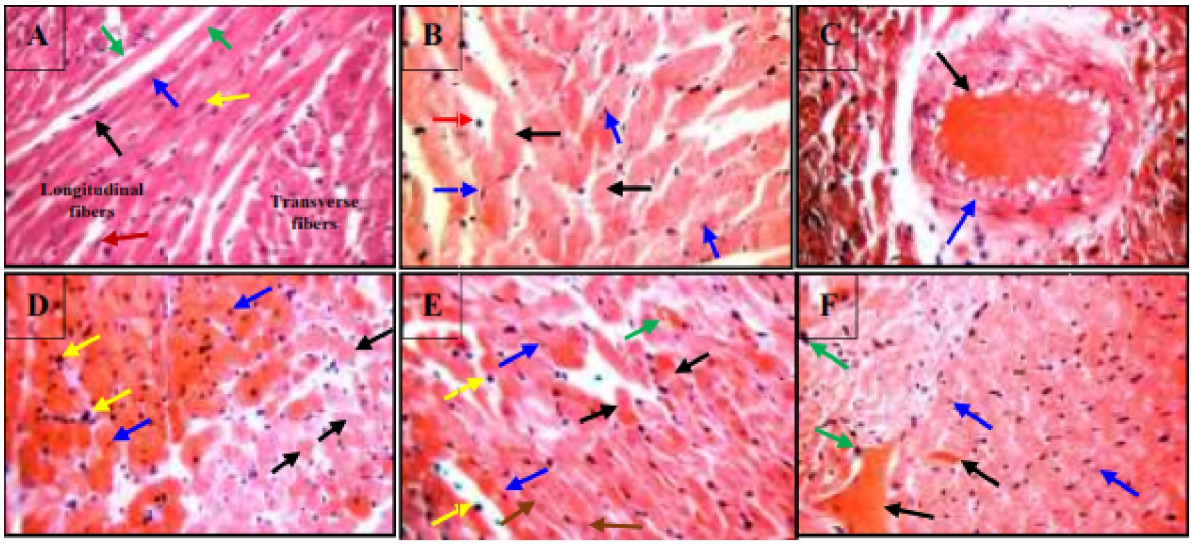

Fig. 3: Photomicrographic sections from the heart of adult albino rats (H & E, X 400). **A-** Control group showing viable cardiac muscle fibers longitudinally and transversely arranged, with distinct cell borders, branching of cardiomyocytes at acute angles, oval centrally located one nucleus (blue arrow) or two nuclei (black arrow), with nuclei having one nucleolus (yellow arrow) or two prominent nucleoli (red arrow) and apparent cross striations (green arrows). **B-** Epinephrine-treated group showing totally necrotic cardiac muscle fibers with pale eosinophilic cytoplasm, indistinct cell borders and pyknotic nuclei (blue arrows), no nuclei in other muscle fibers (black arrows), and scattered mononuclear inflammatory cells (red arrow). **C-** Epinephrine-treated group showing completely occluded blood vessel with swollen endothelial lining (blue arrow) and thrombus attached to its wall (black arrow). **D-** Ramipril-treated group showing partially viable myocardial tissue, with partially preserved cell borders and pyknotic nuclei (blue arrows); and other muscle fibers showing pale cytoplasm and no nuclei (black arrows), with scattered mononuclear inflammatory cells (yellow arrows). **E-** Rosuvastatin-treated group showing partially viable myocardial tissue with partially preserved cell borders, some normal nuclei (brown arrows) and other pyknotic nuclei (blue arrows), some cardiac muscle fibers has no nuclei (black arrows), scattered mononuclear infiltrate (yellow arrows) with proliferating new blood vessels (green arrow). **F-** Combined rosuvastatin- and ramipril-treated group showing more viable myocardium with more viable nuclei (blue arrow), proliferating blood vessels (black arrows) and scattered mononuclear inflammatory infiltrate (green arrows).

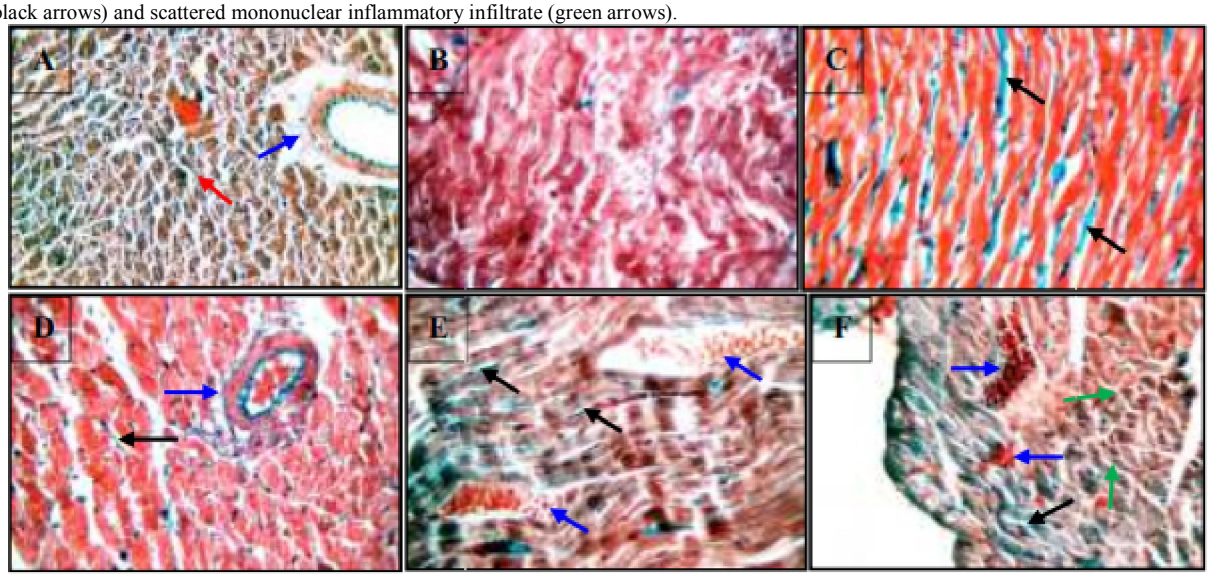

Fig. 4: Photomicrographic sections from the heart of adult albino rats (Masson's trichrome stain, X 400). **A-** Control group showing a normal coronary artery with normal collagen distribution in blood vessel wall (blue arrow) and very thin collagen layer are seen in between muscle fibers (green arrow). **B-** Epinephrine-treated group showing an area of active stage of myocardial infarction with severely distorted cardiomyocytes and lost cell borders. **C-** Epinephrine-treated group showing healed MI with thick irregular collagen layer in between cardiac muscle fibers (black arrows) and indistinct cell borders. **D-** Ramipril-treated group partially preserved cell borders and thin irregular collagen fibers around thrombosed blood vessel (blue arrow) and in between cardiac muscle fibers (black arrow). **E-** Rosuvastatin-treated group showing partially viable myocardial tissue with partially preserved cell borders, thin irregular collagen fibers around proliferating thin-walled angiogenic blood vessels (blue arrows) and mildly thick collagen fibers in between cardiac muscle fibers (black arrows). **F-** Combined rosuvastatin- and ramipril-treated group showing more viable myocardial tissue with preserved cell borders, thin irregular collagen fibers around proliferating thin-walled blood vessels (blue arrows) and in between cardiac muscle fibers (black arrow); and other cells show no collagen fibers in between them (green arrows).

It is well known that catecholamines are released from the sympathetic nerve endings and adrenal medulla under stressful situations. Initially, these hormonesproduce beneficial effects on the cardiovascular system to meet the energy demands of various organs in the body, and their actions on the heart are primarily mediated through the stimulation of β-adrenergic receptors, G protein, adenylate cyclase, and cAMP system in the myocardium***[Mamoojee et al., 2016]***. However, prolonged exposure of the heart to high levels of catecholamines results in coronary spasm or occlusion, arrhythmias, contractile dysfunction, cell damage, myocardialnecrosis, hemorrhage and fibrosis ***[Takizawa et al., 2007]***.

In the pesent work, the death rate among the treated rats was very high especially the male rats. Approximately, all epinephrine-treated male rats died. This is due to two reasons: ***Firstly***,***Tranter et al., (2014)*** stated that, estrogen is a well-known protective factor from occurrence of myocardial ischemia. This is evident clinically as women before menopause has a lower incidence of MI compared to men and this incidence increases in women after menopause. Furthermore, there is a laboratory evidence that estrogen supplementation protects against MI; and on the other hand, ovariectomy increases epinephrine-induced mortality in a rat MI model.

Secondly, ***Suthahar et al., (2017)*** mentioned that, hearts from male animals display a higher number of infiltrating M_1 macrophages (M_1 macrophages are responsible for the inflammatory process of MI) than female hearts. Therefore, male rats are expected to develop more severe form of myocardial infarction.

The specimens in all groups in this study were stained by Hematoxylin and Eosin, and Masson's trichrome stain for histopathological examination. Hematoxylin and Eosin stain is the best stain that can demonstrate the acidophilic and basophilic components of the cell, and Masson's trichrome was used to demonstrate fibrous tissue and collagen fibres.

In the present study, induction of MI was done by once daily subcutaneous injection of (2 mg/kg) epinephrine for 2 days in adult albino rats. This dose was also given by ***[Huda and Akhter, 2014; Parvin and Akhter, 2008]***to induce MI. We observed that death rate of rats in this group was very high compared to other treated groups. Moreover, there was an obvious increase in body weight in rats in this group. These results were observed previously by ***[Grimm et al., 1998; Muders et al., 1999]*** and they attributed these findings to the development of heart failure due to deterioration of myocardial function after MI.

The results of this study clarified that epinephrine-induced MI lesions visualized on light microscopy as sub-epicardial, sub-endocardial and intramyocardial necrosis in the form of focal cardiomyocyte necrosis or contraction band necrosis (group of adjacent cardiomyocytes). There were also ill-defined or lost cell borders, pycnotic or absent nuclei, pale cytoplasm and scattered inflammatory cells. These findings are in correlation with the previous experimental studies ***[Ferrans et al., 1970; Huda and Akhter, 2014; Muders et al., 1999; Parvin and Akhter, 2008]*** who reported, similar structural changes in animals receiving epinephrine.

Moreover, the present study revealed that epinephrine caused intramyocardial hemorrhage and also coronary artery occlusion (thrombosed artery). These results are in agreement with ***[Bayir et al., 2012; Rahmathulla and Lakshmi Devi, 2013]***. They stated that, epinephrine is the most potent catecholamine which causes myocardial hemorrhage and coronary artery spasm and and sometimes occlusion.

Furthermore, Masson's trichrome stained sections of MI group showed thick irregular bands of fibrous tissue inbetween cardiomyocytes which replaced the necrotic cells. These findings denote myocardial fibrosis and were observed by ***[Huda and Akhter, 2014; Parvin and Akhter, 2008]***.

In the present study, another group of rats were given (3 mg/kg/day) ramipril for 30 days starting two days after induction of MI by epinephrine. Ramipril is an ACE inhibitor used occasionally for treatment of hypertensive patients, in stroke patients and also in MI patients. We observed that, the death rate and also the increased body weight of rats in this group was diminshed in comparision with the MI group. These results were mentioned by ***[Bayir et al., 2012; Keles et al., 2009]*** and they attributed these findings to the beneficial effect of ramipril on heart; and hence, prevention of more development of heart failure.

This study also revealed that, sections from ramipril-treated rats showed partially preserved cell borders, less eosinophilic cytoplasm and increased signs of viable myocardium in comparision with the MI group. These findings were mentioned also by ***[Grimm et al., 1998; Safari et al., 2012]*** who attributed these findings to anti-inflammatory, anti-oxidative and vasodilator effects of ramipril.

Exclusively in ramipril-treated group, sections showed the three stages of inflammation which occurs in MI; an area of healed infarction (healing stage), covered by thick fibrinous layer (stage of active inflammation), with intervening area of edema in between the previous two areas (late stage of inflammation). These findings may be attributed to the

anti-apoptotic and anti-inflammatory effects of ramipril leading to delay in the inflammatory process ***[Bayir et al., 2012]***.

Moreover, angiogenic blood vessels are not observed in ramipril-treated rats denoting that ramipril has no role in enhancing angiogenesis. This is in agreement with ***[Bayir et al., 2012; Keles et al., 2009; Wu et al., 2009]*** who stated that ramipril has an equivocal effect on angiogenesis which mean it does not enhance nor inhibit angiogenesis.

Furthermore, Masson's trichrome sections in this group showed thin irregular collagen fibers in between cardiomyocytes but less obvious compared to MI group. This is in agreement with ***[Bayir et al., 2012; Keles et al., 2009; Safari et al., 2012; Wu et al., 2009]*** who attributed these findings to blockage of angiotensin II which has a pro-fibrotic effect, resulting in minor form of fibrosis.

In the present study, another group of rats were given (10 mg/kg/day) rosuvastatin "high dose of rosuvastatin" for 30 days starting two days after induction of MI by epinephrine. Rosuvastatin is a new member of HMG CoA reductase inhibitors and known as "super-statin" due to its high potency -compared to other members- in prevention and treatment of dyslipidemia and in ischemic conditions such as stroke, ischemic limbs and MI. In accordance with ***[Ikeda et al., 2003]*** and ***[Variya et al., 2015]***; we revealed that, the death rate and also the increased body weight of rats in this group was diminshed in comparision with the epinephrine- and ramipril-treated rats.

This study demonstrated that, sections from rosuvastatin-treated rats showed partially preserved cell borders, less eosinophilic cytoplasm and increased signs of viable myocardium in comparision with the epinephrine- and ramipril-treated rats. These findings were mentioned also by ***[Huang et al., 2015; Ikeda et al., 2003; Variya et al., 2015]*** who attributed these findings to anti-inflammatory, anti-oxidative, anti-hyperlipidemic and endothelial fixation effects of rosuvastatin.

Ikeda et al., (2003) mentioned that, the cardioprotective effect of rosuvastatin on myocardium is strongly evident even in non-hypercholesterolemic rats. In addition, many clinical trials on ischemic patients were done and clarified the previous conclusion ***[Goldstein and Brown, 2015; Jones et al., 2003; Ong, 2005]***.

Moreover, sections from rosuvastatin-treated rats showed marked angiogenesis (in the form of channels carring blood and lined only by endothelial cells which rest on basement membrane). These findings proved that rosuvastatin has a great role in enhancing angiogenesis. Our results were in agreement with the results obtained by ***[Huang et al., 2015; Variya et al., 2015]***.

In addition, ***Zaitone and Abo-Gresha, (2012)*** observed enhanced angiogenesis in MI rats treated with rosuvastatin and attributed this to increased expression of VEGF and iNOS. Furthemore, ***Zhou et al., (2013)*** clarified that, rosuvastatin-treated rats showed enhanced angiogenesis via eNOS-dependent mobilization of endothelial progenitor cells from bone marrow to the infarcted area.

Furthermore, Masson's trichrome sections in rosuvastatin-treated group showed mildely thick irregular collagen fibers in between cardiomyocytes, which were less obvious compared to epinephrine-treated rats but more obvious compared to ramipril-treated rats. Also, there were clearly visualised angiogenic blood vessels in these sections. These findings were in agreement with ***[Huang et al., 2015; Variya et al., 2015]*** who attributed these findings to anti-inflammatory, anti-platelet, angiogenic and endothelial modulator effects of rosuvastatin on myocardium, resulting in minor form of MI and enhanced angiogenesis.

In disagreement with our findings, ***Weis et al., (2002)*** stated that, low dose rosuvastatin (0.1-1 mg/kg) can enhance angiogenesisand not the high dose (5-10 mg/kg).

In the present study, another group of rats were given (10 mg/kg/day) rosuvastatin and together with (3 mg/kg/day) ramipril for 30 days starting two days after induction of MI by epinephrine. In accordance with ***Cattaneo and Remuzzi, (2005)***, we revealed that, the death rate and also the increased body weight of rats in this group was decreased and achieved the best results in comparision with other treated groups. Moreover, this group achieved the best results among the treated rats; as its sections showed increased signs of viable myocardium in comparision with other treated groups.

Furthermore, ***Han et al., (2012)***made a coronary angiographycomparision between patients with coronary artery disease (CAD) receiveing combined rosuvastatin and ramipril and other patients receiving only rosuvastatin. They demonstrated that atheroma volume become greatly reduced in patients receiveing combined rosuvastatin and ramipril compared to patients receiving rosuvastatin alone. They attributed this difference mainly to the anti-inflammatory mechanism of both agents.

Concerning angiogenesis, this group showed enhanced angiogenesis exactly like angiogenesis in the rosuvastatin-treated rats. This confirmes our previous observation that, ramipril has no effect on angiogenesis (never enhance nor inhibit angiogenesis).

Furthermore, Masson's trichrome sections in combined rosuvastatin- and ramipril-treated group

showed, thin irregular collagen layer in between cardiomyocytes but less obvious compared to rats in other treated groups. These findings denote that this group achieved the best results among all treated groups; or in others words, this group had the minor form of MI and fibrosis compared to other groups.

5. Conclusion

In conclusion, the outcome of this study shows that the epinephrine in large doses has a toxic effect on myocardium and produces MI and fibrosis with their deleterious effects, especially, heart failure. These lesions were greatly ameliorated by concomitant administration of both rosuvastatin and ramipril. So, it is advisable in patients with MI to receive both agents together.

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