Original Article

Platelet-Rich Plasma Attenuates Isoproterenol-Induced Myocardial Injury in Adult Male Albino Rat: Histological and Immunohistochemical Study

Marwa Mohammed Soliman, Maha Mostafa Ahmed Zakaria, Hany Shawky Nadim, Walaa Atef Hassan El-Fakharany, Marian Mokhtar Shokry and Ahmed Samir Sadek

Department of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, Egypt

ABSTRACT

Introduction: Myocardial infarction (MI) is a global burden that implies on quality of life. Platelet-rich plasma (PRP) is an autologous source of growth factors that is recently suggested due to its healing properties. Isoproterenol (ISO) is a β-agonist drug used as experimental model of myocardial injury through cardiac hyperactivity and production of reactive oxygen species.
Aim of Work: To investigate the role of PRP in preservation of cardiac tissue and enhancing healing in isoproterenol (ISO) induced myocardial injury.
Materials and Methods: 48 adult male albino rats were used as follows; Control group (N=18), Myocardial injury group (N=18) and Myocardial injury with PRP group (N=12). ISO was administered as a single subcutaneous dose (67 mg/kg) and PRP was injected intracardiac after 12 h from ISO injection. Hearts were harvested at 7 days and 21 days and left ventricular sections were stained for histological and immunohistochemical study.
Results: Isoproterenol treated sections showed necrosis and apoptosis of myocardial fibers with loss of striations. Interstiuim showed mononuclear cellular infiltration and significant increase in collagen deposition. Moreover, blood vessels showed congestion and hemorrhage. Examination of PRP treated sections showed restoration of normal architecture of myocardial fibers. There was a statistically significant decrease in apoptosis and necrosis of myocardial cells. Also, interstitium showed an apparent decrease in mononuclear cellular infiltration and hemorrhage. These findings were observed after one week of PRP administration and became more evident after three weeks. Meanwhile, collagen deposition showed highly significant decrease in PRP treated group only after three weeks in comparison to ISO only treated group.
Conclusion: PRP was found to decrease myocardial death and accelerate healing. PRP can be considered a simple, economically feasible and favorable strategy in management of myocardial injury.

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Key Words: Myocardial infarction, isoproterenol, platelet-rich-plasma.

Corresponding Author: Marwa Mohammed Soliman, MD, Department of Anatomy and Embryology, Faculty of Medicine, Ain Shams University, Egypt, **Tel.**: +20 10 1320 2216, **E-mail:** marwa.soliman@med.asu.edu.eg

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INTRODUCTION

Myocardial infarction (MI) is a serious cardiovascular disease (CVD) that occurs due to imbalance between the coronary blood flow and myocardial demands[1].

In Egypt, the prevalence of CVD is estimated to be 8.3% as reported by the National Hypertension Project. CVD is further exaggerated by the higher cost of surgical procedures and prolonged medical treatment, in addition to disabilities and rehabilitation requirements needed[2]. WHO stated that the burden of MI and strokes reached about 32.4 million cases worldwide every year[3]. Moreover, it is suggested that the CVD mortality will progress from 30% to 33% worldwide by 2030[4].

Many risk factors contributed to developing MI ranging from non-adjustable factors as family history, sex and age to adjustable factors as smoking, alcohol use, lack of physical activity and obesity. Moreover, other pathological conditions are listed as risk factors like hypertension and diabetes mellitus[5].

MI may be presented with variable but non-specific symptoms as dyspnea, chest pain, shoulder and jaw discomfort, and even epigastric. Moreover, some patients may not exhibit any symptoms at all, so cardiac imaging, ECG, and biomarkers detection are important for diagnosis of MI[6].

Clinically, MI is classified into type 1 MI: happens by disrupted plaque in atherosclerosis with thrombotic coronary artery disease and type 2 MI: caused by inequity between oxygen demands and supply of myocardial tissue[7].

Management of MI varies according the ischemic profile of the patient like ST segment elevated MI (STEMI), non-STEMI, unstable angina, or non-ischemic disorder in addition to, patient assessment for the risk of

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thrombosis or repeated attacks[8]. Drugs are used include aspirin, statin, nitroglycerin, Oxygen if needed, morphine or beta blockers. Cardiologists add another anti-platelet agent to aspirin or an anticoagulant depending on the risk of thrombosis and the chosen management procedure for the patient[9]. Interventional techniques are widely used such as; Percutaneous Coronary Intervention (PCI) with emergency intravenous fibrinolytic therapy and Coronary artery bypass grafting (CABG)[10].

Researchers worldwide have been studying numerous novel techniques in management of myocardial injury such as., Injectable cardiac tissue like mesenchymal, embryonic or, bone marrow-derivatives of hematopoietic stem cells[11]. Injectable biomaterials were also tried like nanofibers releasing growth factors[12]. Other immunomodulatory experiments were tried to oppose the inflammatory cascade in myocardial infarction such as; complement cascade inhibition[13], neutrophil recruitment inhibition[14]. Also regulatory T-lymphocytes (Tregs) modulation showed cardioprotective property[15].

Experimental myocardial injury has been tried in many studies. Isoproterenol hydrochloride (ISO) is 3,4-Dihydroxy-α- [isopropyl amino methyl] benzyl alcohol hydrochloride, a synthetic catecholamine that causes myocardial injury by acting as a Ɓ-adrenergic agonist[16]. Moreover, oxidative stress and free radicals' formation were reported[17].

Platelet-Rich Plasma (PRP) was introduced as a possible healing promoting agent that attracted many researchers as a safe and financially accepted method and used in treatment of many conditions. It has a higher concentration of autologous growth factors such as; vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF-β1), and platelet-derived growth factor (PDGF), and[18].

However, there is no sufficient research data to support the effect of PRP on the healing of myocardial tissue following drug-induced myocardial injury.

This study aimed to observe the potential role of PRP in decreasing cellular death and enhancing healing cascade in acute myocardial injury.

MATERIALS AND METHODS

Drugs

Isoproterenol hydrochloride 98% (ISO) was obtained from Sigma-Aldrich chemicals company. It was injected in the right thigh of the animal as a single subcutaneous dose (67 mg /kg) dissolved in phosphate buffered saline (PBS)[19].

Platelet-rich plasma preparation

Anesthesia of animals was performed by intraperitoneal pentobarbital injection 60 mg / kg; then whole blood was extracted from the retro-orbital plexus of vessels with a capillary tube[20]. Blood was immediately deposited in sodium citrate test tube and centrifuged at 200 g for 15 minutes, dividing the sample into three portions: a bottom red fraction made up mainly of red blood cells; an intermediate yellow-straw portion (buffy coat), which contains the serum component; and a top blood plasma fraction. The buffy coat was pipetted out of the top fraction, which was then spun again at 500 g for ten minutes. The bottom content composed of PRP was pipetted[21]. After 12 h from ISO injection, 100 µl of PRP were injected intracardiac using subxiphoid approach; the xiphoid process of the sternum and the left costo-sternal angle were marked, and the needle was inserted one cm to the left of the xiphoid process and aimed toward the left shoulder[22].

Experimental rats

Following permission from CARE; the Animal Research Ethics Committee at Ain Shams University's Faculty of Medicine coded (M.D 31/2020 ⁄ 29/1/2020). Forty-eight adult male albino rats weighing 200- 250 gm were housed at Ain Shams Faculty of Medicine's animal household of medical research center. Each three rats were kept in medium sized stainless-steel cage, Rats were given regular dark\ light cycles and a daily diet with unrestricted access to water. Regular cage cleaning was done. Animals were housed for seven days prior to the experiment to give them time to acclimatize. They were divided into three groups:

Group I (control group): It included 18 rats subdivided into three subgroups:

- Group IA: Six rats that received saline injection in the right thigh and were sacrificed after 12 h.
- Group IB: Six rats that received intracardiac injection of Phosphate Buffered Saline (PBS) and were sacrificed after seven days.
- Group IC: Six rats that received intracardiac injection of PBS and were sacrificed after 21 days.

Group II (Myocardial injury group): It included 18 rats that received a single dose (67mg/kg) of subcutaneous injection of ISO (19). They were subdivided into three subgroups:

- Group IIA: Six rats were sacrificed after 12 h from injection.
- Group IIB: Six rats were sacrificed after seven days from injection.
- Group IIC: Six rats were sacrificed after 21 days from injection.

Group III (Myocardial injury with PRP treatment group): It included 12 rats that received ISO. After 12 h from ISO injection, 100 µl of PRP were injected intracardiac as described. Rats were subdivided into two subgroups:

- Group IIIA: Six rats were sacrificed after seven days from PRP injection.

- Group IIIB: Six rats were sacrificed after 21 days from PRP injection.

Rats had their hearts extracted at the end of the experiment while they were all under pentobarbital sodium anaesthesia. Left ventricle samples were preserved in 10% neutral-buffered formalin solution for a week before being turned into paraffin blocks. For the histological analysis, 5 m paraffin slices were employed.

Vital staining of myocardium

Trypan blue dye was purchased from Sigma-Aldrich chemicals company. Rats were given an intramyocardial injection of the dye containing 5 mg in 0.5 cc of a 1% solution through subxiphoid approach. Hearts were removed five minutes after injection and preserved for two hours in Heidenhain's Susa mixture (NaC10.5, acetic acid 4, formalin 20, corrosive sublimate 5, trichloracetic 2, aq. 80). The tissues were then quickly dehydrated in several alcohol changes over the course of four to five hours. After that, the tissues were cleansed in chloroform and immersed in paraffin. Left ventricle sections of 30-micron thickness were cut and examined without counterstain. Dead tissues or cells that have the colour blue (Trypan Blue)[23].

Tissue preparation for light microscopy

The dehydrated fixed left ventricular tissues were immersed in paraffin, divided into 4 μm sections, and stained with Phosphotungstic acid-hematoxylin (PTAH), Masson's trichrome stain, and hematoxylin and eosin (H&E). An Olympus light microscope with an automatic digital photomicrographic camera system was used to examine slides[24].

Tissue preparation for immunohistochemical study

Left ventricular sections were cut at a thickness of 5μ, deparaffinized, hydrated in 3% H_2O_2 for five minutes, and rinsed with PBS for fifteen minutes to conduct the caspase 3 immunohistochemical study. The sections were then incubated with avidin-biotin-peroxidase complex (Santa Cruz Biotechnology, Inc., rabbit peroxidase kit; 1h), DAB (3, 3'-diaminobenzidine) solution, and biotin-conjugated goat anti-rabbit IgG (1:200, 1h, room temperature). Instead of the primary antibody, 1 μg/ml of rabbit IgG was added to the reaction as a negative control. Hematoxylin was used as a counterstain to show the brown colour of apoptosis in the sections[25].

Morphometric study & Image analysis

The following parameters were measured using NIH "Image J" computer image analysis software version 1.40g:

- The percentage of cardiac tissue areas per microscopic field stained for collagen fibers deposition with Masson’s trichrome stain.
- The percentage of apoptotic areas for caspase 3 in immune-stained sections.
- The percentage of dead cardiac area in vitally stained sections with trypan blue.

For each microscopic magnification, the software was calibrated in order to convert pixels into micrometres. With the help of a stage micrometre, this was achieved. Adjusting the colour threshold on the primary mask was performed on the measurement area. To calculate the area percentage, the mask's pixel count was divided by the microscopic field's pixel count.

Statistical Analysis

The means of various groups were compared using one-way analysis of variance (ANOVA). Mean + SD was used to calculate the results. The SPSS programme was used to determine the *P-value* with the aid of post-hoc Bonferroni test. The *P-value* used for assessment of the significance of the results was (*P* 0.05 or equal to 0.05 was regarded significant, and *P* 0.001 or equal to 0.001 was regarded highly significant)[26].

RESULTS

Group I (control group)

All of the control subgroups had similar immunohistochemical and histological findings. They were therefore referred to as the control group (G I).

A-Vital staining of myocardium

Trypan blue stained sections showed negative staining denoting viable cells (Figure 1.a).

B- Light microscopic results

H&E-stained sections showed cylindrically branching architecture and anastomosing fibers of myocardium. Cardiac fibers also showed acidophilic sarcoplasm and oval central vesicular nuclei. Blood vessels showed normal appearance. Dark flattened nuclei of fibroblasts were also noticed in endomysium (Figure 1.b).

Sections Masson’s trichrome stained showed minimal fine collagen fibers in interstitium. (Figure 1.c).

Examination of cardiac fibers using PTAH stain showed normal branching architecture with visible cross striations in the cardiac fibers (Figure 1.d).

C-Immunohistochemical results

Heart sections showed negative immunoreactivity for caspase 3 (Figure 1.e).

Group II (myocardial injury group)

A-Vital staining of myocardium

After one-week form induction of injury by ISO (G IIB), trypan blue staining showed numerous stained cells (Figure 3.a).

After three weeks (G IIC), cardiac tissue showed few stained cells (Figure 4.a).

B- light microscopic results

After 12 hours from ISO injection (G IIA), some left ventricular sections were stained with H&E and

examined by light microscope to detect establishment of acute myocardial injury. Cardiac tissue showed disrupted architecture with vacuolations in some cardiac fibers and others showed homogenous deeply acidophilic cytoplasm. Many of the cardiac fibers exhibited pyknotic nuclei. Intersitium of left ventricle showed congested and dilated blood vessels with extravasation of blood cells between cardiac muscle fibers. Mononuclear cellular infiltration in heart sections was noticed (Figures 2.a,2.b).

One week following ISO administration (G IIB), H&E-stained heart sections showed apparently increased degeneration and pyknotic nuclei of cardiac fibers. Extensive mononuclear infiltration was noticed with observed macrophages in cardiac tissue (Figure 3.b). Meanwhile, after three weeks (G IIC), certain areas of myocardium restored architecture with and other areas still vacuolated with large pizzar nuclei. Fibroblasts were observed by their flattened nuclei (Figure 4.b).

Cardiac endomysium as observed by Masson's trichrome staining showed an apparent increase in collagen deposition after one week from ISO injection (G IIB) that was condensed around hemorrhagic areas (Figure 3.c). After three weeks (G IIC), endomysium showed extensive collagen deposition replacing degenerated areas and interstitial collagen fibers extend between myocardial cells of coarse and fine types as well (Figure 4.c).

Cardiac fibers as observed by PTAH stain showed after one week (G IIB) wide spacing and degeneration with loss of cross striations. Contraction band necrosis was noticed in cardiac fibers (Figure 3.d). After three weeks (G IIC), they showed retaining of architecture and striations in certain areas and disruption with loss of striations in others. Apparent hypertrophy of cardiac cells was noticed (Figure 4.d).

C-Immunohistochemical results

Cardiac tissue as detected by caspase 3 showed after one week (G IIB) a strong immunoreactivity with appearance of brown cytoplasmic stained granules (Figure 3.e). Meanwhile, after three weeks (G IIC), immunoreactivity of cardiac tissue was reduced (Figure 4.e).

Group III (myocardial injury with PRP treatment group)

A- Vital staining of myocardium

Revealed apparent decrease in stained non-viable cells either in one-week PRP treated group (G IIIA) or in three weeks (G IIIB) in comparison to untreated groups Trypan blue staining showed fewer stained cells after one week (Figure 5.a). After three weeks, minimal stained cells were detected with restoration of almost normal cardiac architecture (Figure 6.a).

B-light microscopic results

Light microscopic examination of H&E-stained heart sections of Group IIIA showed restoration of branching architecture of cardiac fibers. But some fibers still showed pyknotic nuclei. Fibroblasts recruitment was observed in this group by their flattened nuclei (Figure 5.b). Meanwhile, after three weeks (G IIIB), myocardial fibers showed almost normal architecture with vesicular nuclei and visible striations. Blood vessels showed normal appearance and fibroblasts were also noticed (Figure 6.b).

Cardiac endomysium by Masson's trichrome staining of Group IIIA showed an apparent increase in collagen compared to control sections. In one-week ISO treated group, collagen was more or less alike to that of PRP treated group. (Figure 5.c). After three weeks, decrease in collagen deposition in PRP treated group (G IIIB) compared to ISO only treated group was noticed. Minimal fine collagen fibers were noticed between cardiac cells (Figure 6.c).

Examination of PTAH-stained sections after one week (G IIIA) revealed retaining of architecture and cross striations in certain areas with some non-striated fibers in other areas (Figure 5.d). Meanwhile, after three weeks (G IIIB), most of myocardial fibers retained their branching architecture and striations (Figure 6.d).

C-Immunohistochemical results

Apoptosis as detected by caspase 3 stain showed an apparent decrease after one week & three weeks of PRP administration. In one week (G IIIA), myocardial tissue showed fewer cells still showed immunoreaction (Figure 5.e). Meanwhile, after three weeks (G IIIB), most of the tissue showed negative immunoreactivity with minimal apoptotic cells were stained (Figure 6.e).

Morphometric results and statistical analysis

Mean area percentage of stained non-viable myocardial cells in different groups (Table 1, Chart 1).

Mean area percentage of stained collagen fibers between different groups (Table 2, Chart 2)

Mean area percentage of immunoreactive cells for apoptosis between different groups (Table 3, Chart 3).

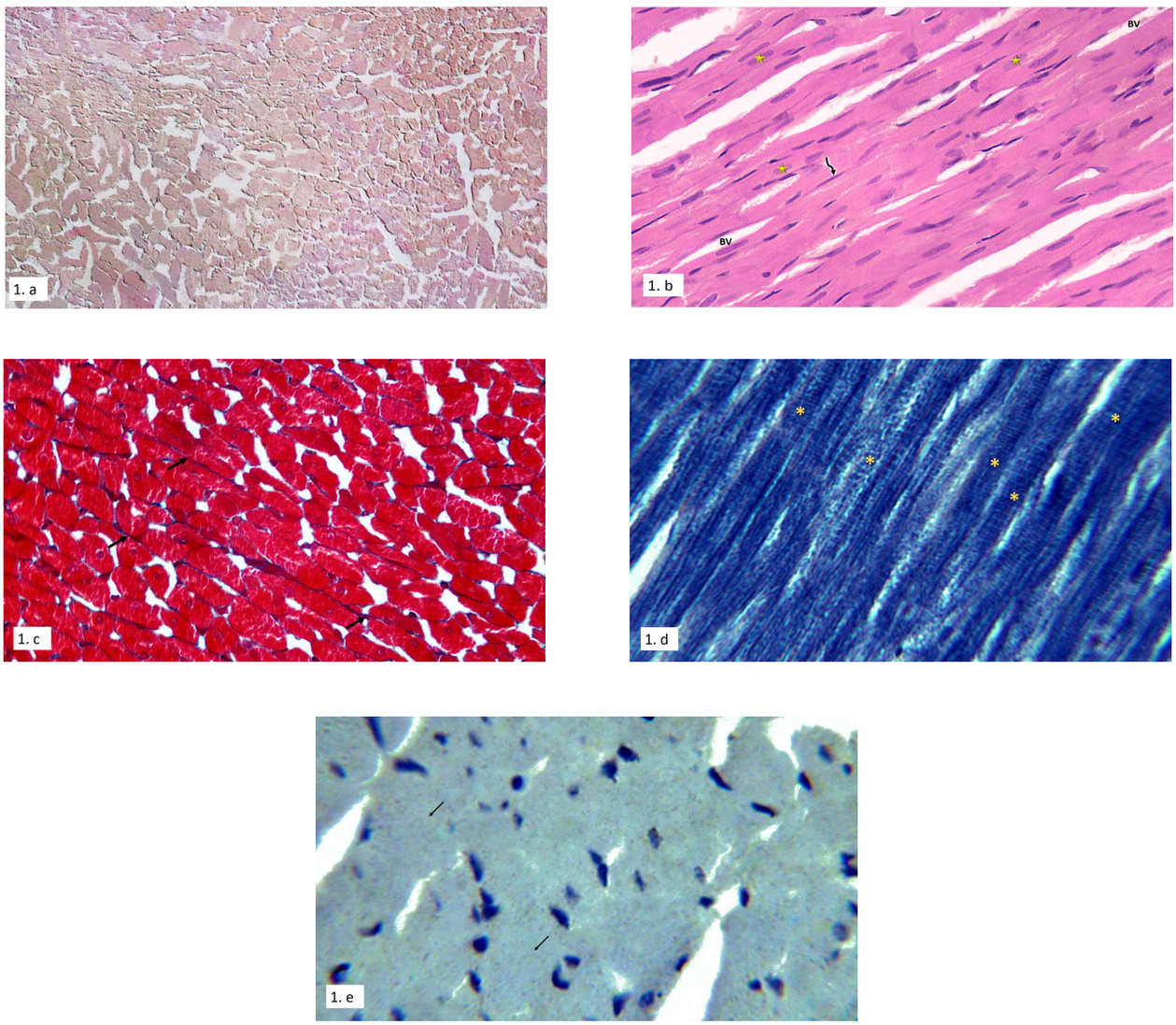

Fig. 1: Photomicrographs of left ventricular myocardial sections in control group (G I) showing: (a): viable cardiac tissue with negative reaction to vital staining (trypan blue x400). (b): myocardial fibers are cylindrically branching with acidophilic sarcoplasm and visible striations. Oval vesicular nuclei (yellow stars) and capillaries are seen between cardiac fibers (BV). Notice the flattened nuclei of interstitial fibroblasts (curved arrow) (H&E x400). (c): cardiac interstitium with minimal collagen fibers (black arrows) (Masson's trichrome x400). (d): cardiac muscle fibers are branching and anastomosing with visible cross striations (black asterix). (PTAH x1000). (e): cardiac myocytes with negative immunoreactivity (arrows) (caspase x1000).

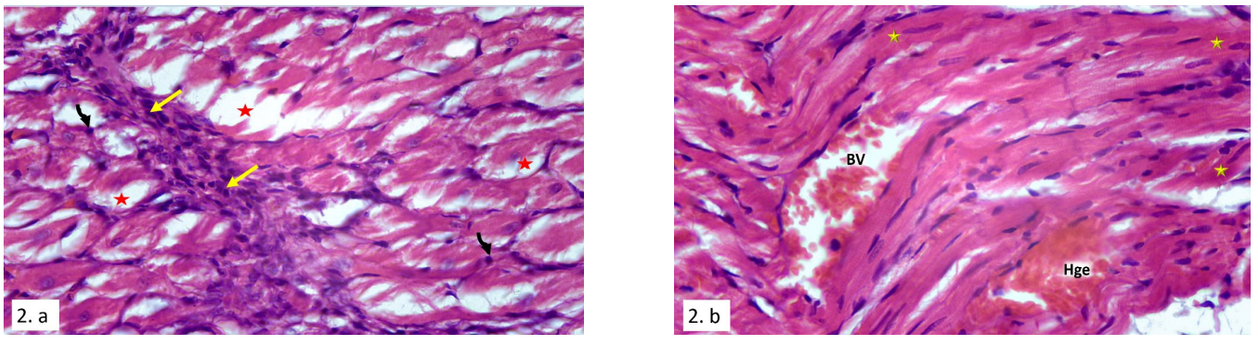

Fig. 2: Photomicrographs of H&E stained left ventricular myocardial sections 12 h. after ISO injection (G IIA) showing: (a): cardiac fibers with disrupted architecture, vacuolation and degeneration (red stars). Some Cardiac fibers show pyknotic nuclei (curved arrows). Mononuclear infiltration was noticed in cardiac interstitial tissue (yellow arrows). (b): myocardium shows areas with deeply homogenous acidophilic cytoplasm (yellow stars). Blood vessels are dilated and congested (BV) with extravasation of blood in cardiac tissue (Hge) (H&E x400).

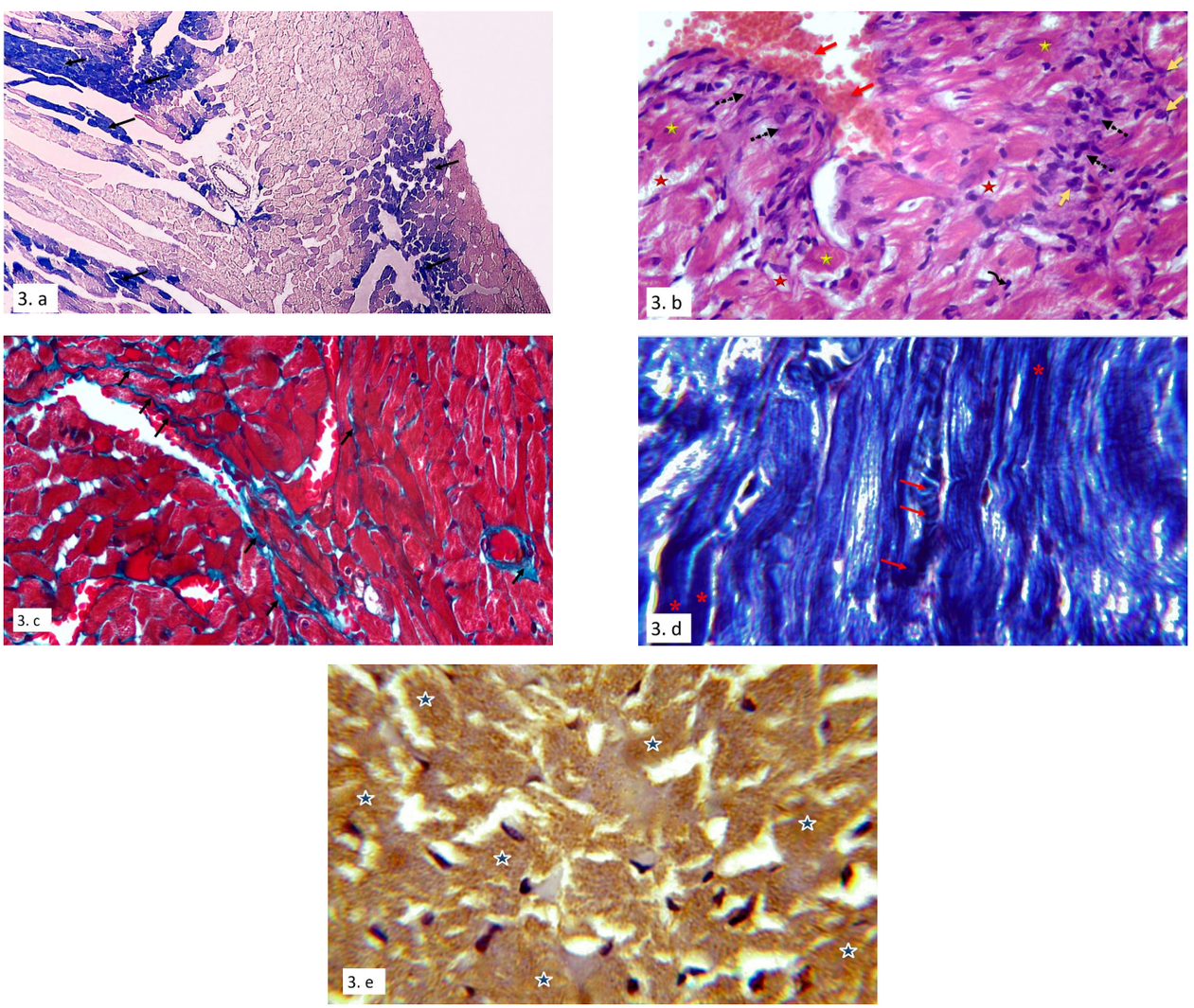

Fig. 3: Photomicrographs of left ventricular myocardial sections in one week ISO treated group (G IIB) showing: (a): myocardial areas & scattered cells of stained non-viable myocardial cells (black arrows) (trypan blue x100). (b): cardiac myocytes with disrupted architecture and vacuolations (red stars). Some cardiac myocytes have pyknotic & karyolitic nuclei (curved arrow). The interstitium of heart shows hemorrhage (red arrows) & extensive mononuclear infiltration (dashed arrows). Noticed cells with large nucleus and foamy cytoplasm (macrophages) (yellow arrows) (H&E x400). (c): cardiac endomysium with dense irregular collagen fibers (black arrows) (Masson's trichrome x400). (d): cardiac fibers with disrupted architecture and loss of cross striations (red asterix). Notice contraction band necrosis (red arrows) (PTAH x1000). (e): cardiac myocytes with positive immune reaction in the cytoplasm (stars) (caspase x1000).

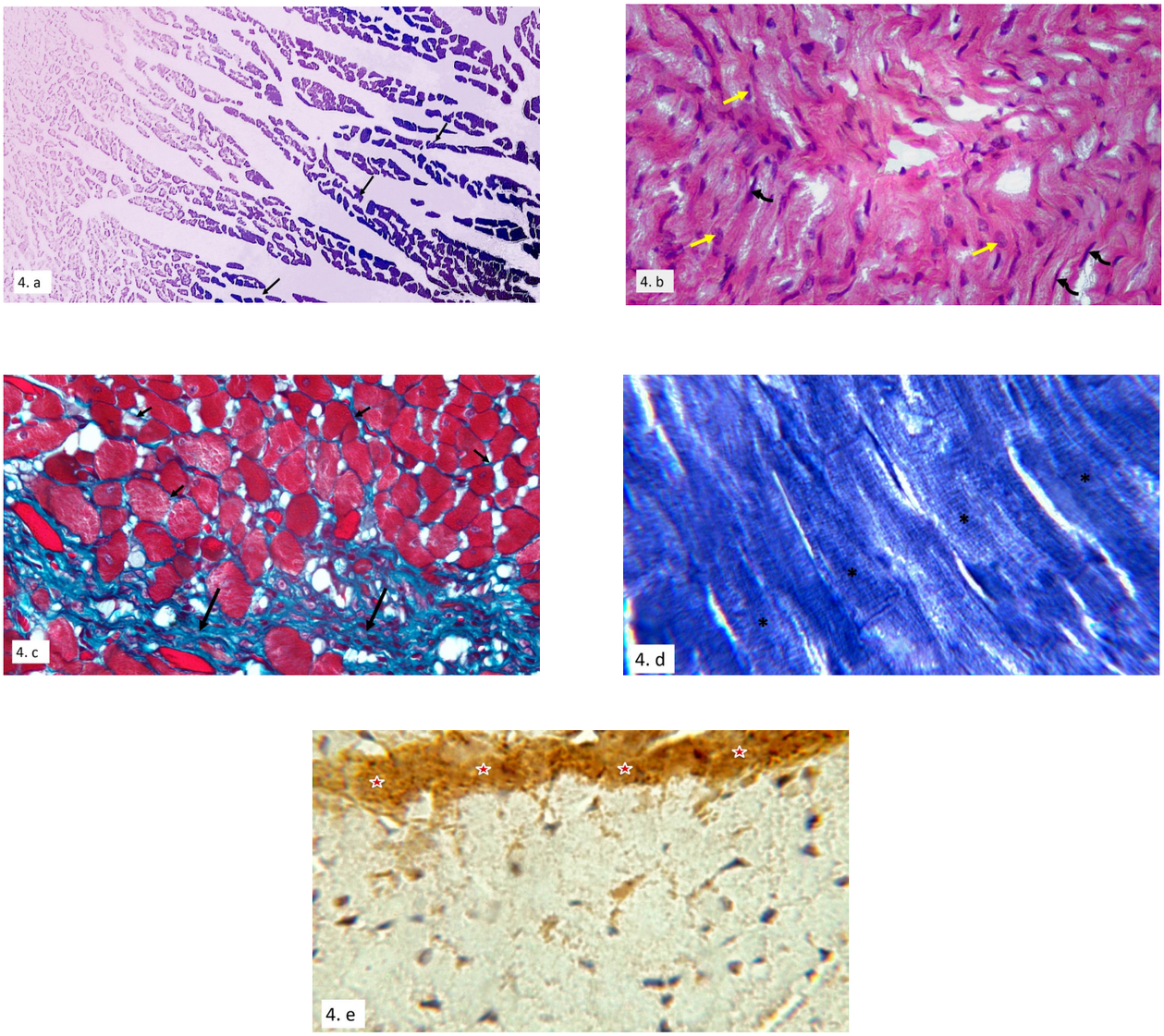

Fig. 4: Photomicrographs of left ventricular myocardial sections in three weeks ISO treated group (G IIC) showing: (a): myocardial areas of stained non-viable myocardial cells (arrows) (trypan blue x400). (b): cardiac myocytes with large pizzar shaped nuclei (yellow arrows). Numerous fibroblasts can be observed by their flattened nuclei (curved arrows) (H&E x400). (c): cardiac interstitium with apparent increase in deposition of collagen fibers (black arrows) (Masson's trichrome x400). (d): Most of cardiac fibers regain their cross striations (black asterix). Notice apparent hypertrophy in myocardial fibers (PTAH x1000). (e): cardiac tissue with few positive stained myocardial fibers (stars) (caspase x1000).

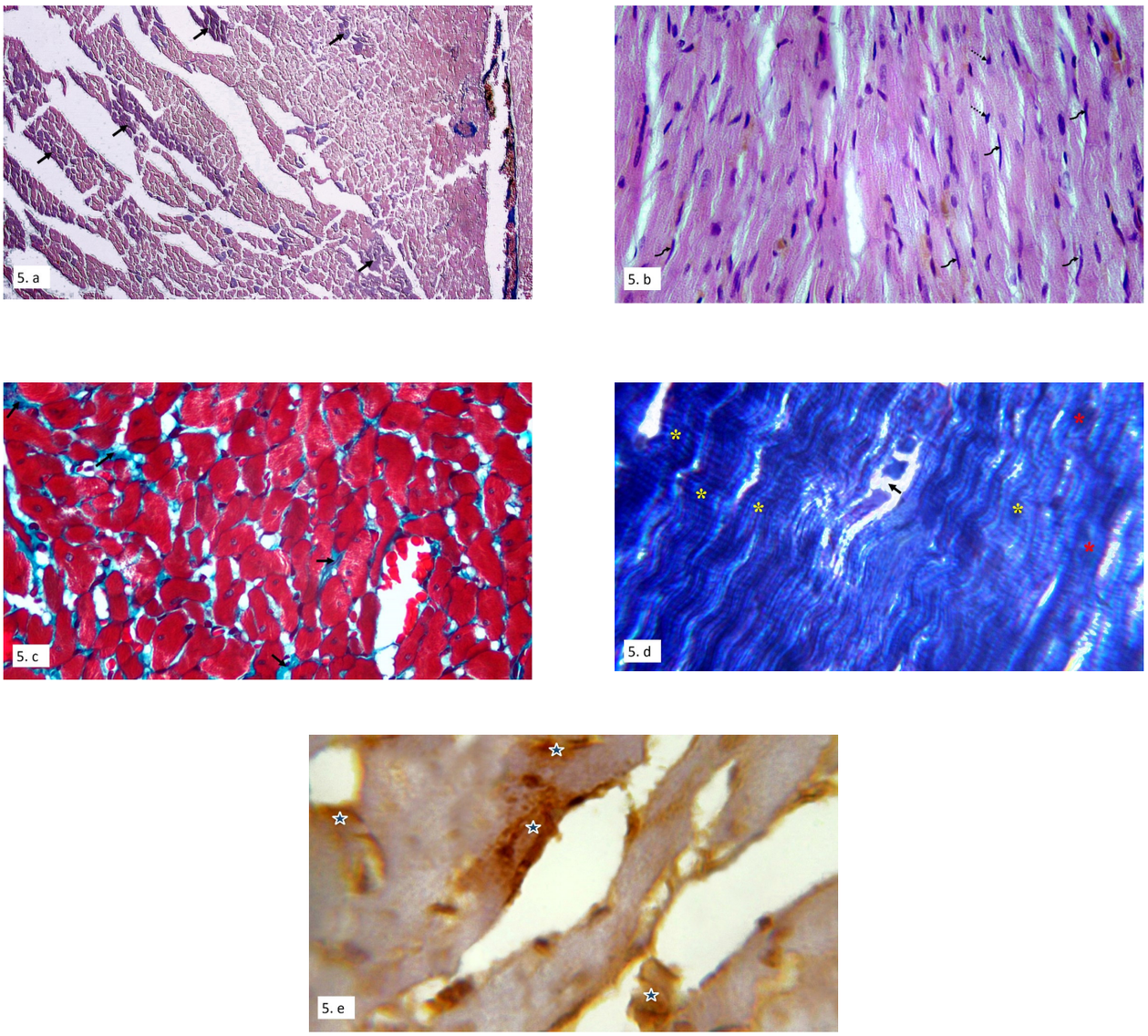

Fig. 5: Photomicrographs of left ventricular myocardial sections in one week PRP-treated group (G IIIA) showing: (a): myocardial tissue with few positive stained cardiac cells (black arrows) (trypan blue x400). (b): cardiac fibers with branching and anastomosing architecture. Few fibers still have pyknotic nuclei (dashed arrows) with the loss of striations. Notice appearance of flattened nuclei of fibroblasts (curved arrows) (H&E x400). (c): cardiac intersitium with fine collagen fibers (black arrows) (Masson's trichrome x400). (d): few disconnected fibers (black arrow) with loss of striations (red asterix). Most of the fibers show striations (yellow asterix) (PTAH x1000). (e): cardiac tissue with positive immunoreactivity of some of the cardiac cells (stars) (caspase x1000).

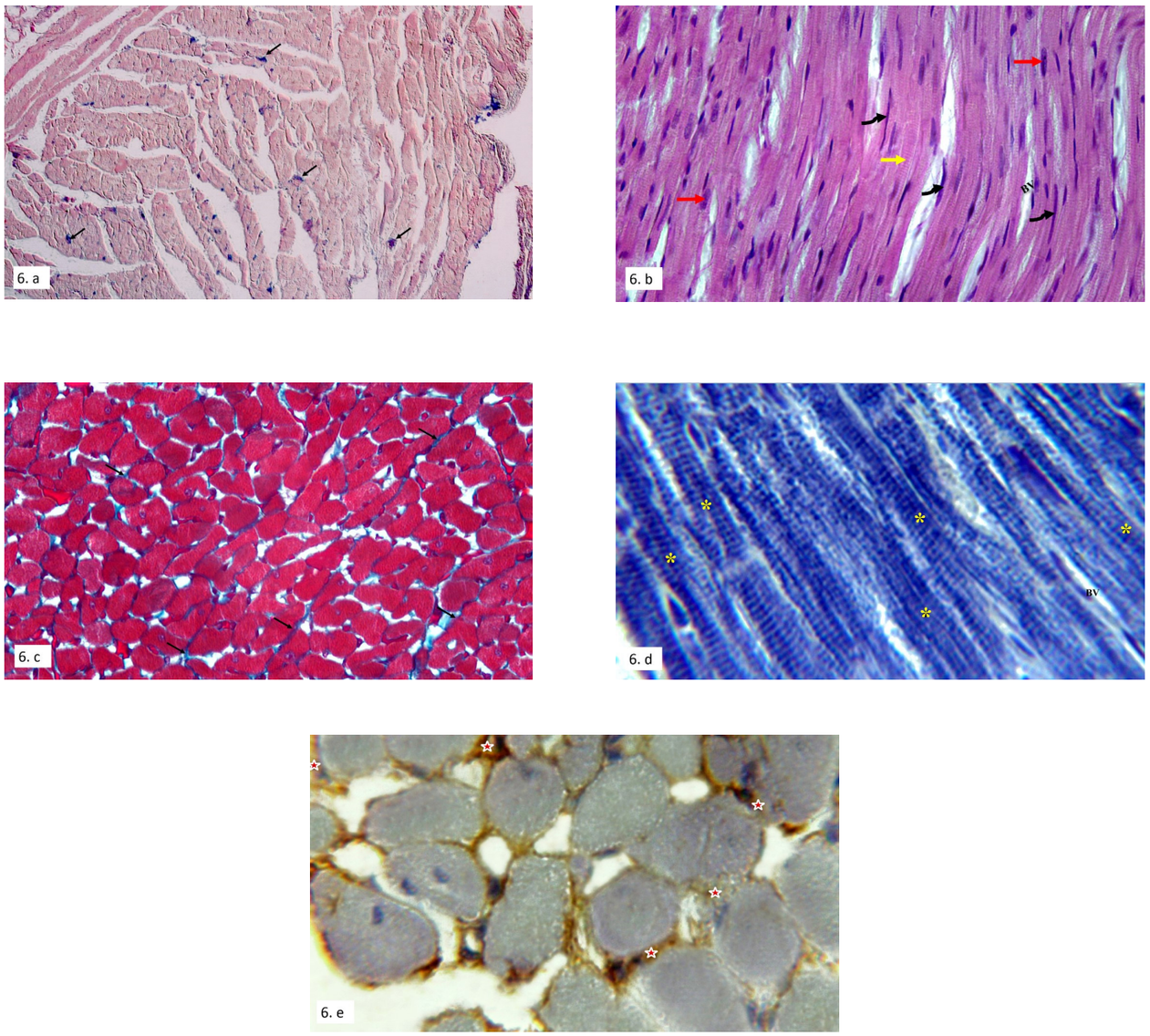

Fig. 6: Photomicrographs of left ventricular myocardial sections in three weeks PRP-treated group (G IIIB) showing: (a): cardiac tissue with very few stained myocardial cells (black arrows) (trypan blue x400). (b): cardiac tissue retained normal architecture in most areas with visible striations (yellow arrow) and vesicular nuclei (red arrows). Notice normal appearance of blood vessels (BV) and flattened nuclei of fibroblasts (curved arrows) (H&E x400). (c): cardiac intersitium with minimal fine collagen fibers (black arrows) (Masson's trichrome x400). (d): cardiac fibers retained the branching normal architecture and cross striations in most of areas (yellow asterix) with normal appearance of blood vessels (BV) (PTAH x1000). (e): cardiac tissue with minimal immunoreactivity of the interstitial cells (stars) (caspase x1000).

Table 1: Mean area percentage of stained non-viable myocardial cells in different groups

	Control Group	MI One week	PRP One week	MI Three weeks	PRP Three weeks
Mean ± standard deviation	0.26 ± 0.07	15.26 ± 4.02	2.95 ± 0.76	12.62 ± 3.78	0.74 ± 0.27
P- value			(0.009)[1]		(0.002)[2]

(P)[1] = significant decrease compared to MI one week group.
(P)[2]= significant decrease compared to MI three weeks group.

Table 2: Mean area percentage of stained collagen fibers between different groups

	Control Group	MI One week	PRP One week	MI Three weeks	PRP Three weeks
Mean ± standard deviation	7.89 ± 2.16	21.61 ± 4.67	13.99 ± 3.76	24.14 ± 5.69	10.21 ± 3.06
P- value			0.04)[1] (0.074)[2]		(0.0006)[3]

(P)[1] = significant increase compared to control group.
(P)[2]= non-significant decrease compared to MI one week group.
(P)[3]= highly significant decrease compared to MI three weeks group.

Table 3: Mean area percentage of immunoreactive cells for apoptosis between different groups

	Control Group	MI One week	PRP One week	MI Three weeks	PRP Three weeks
Mean ± standard deviation	0.03 ± 0.009	31.28 ± 9.19	12.15 ± 3.29	21.55 ± 6.79	2.02 ± 0.45
P- value			(0.004)[1]		(0.0001)[2]

(P)[1] = significant decrease compared to MI one week group.
(P)[2]= highly significant decrease compared to MI three weeks group.

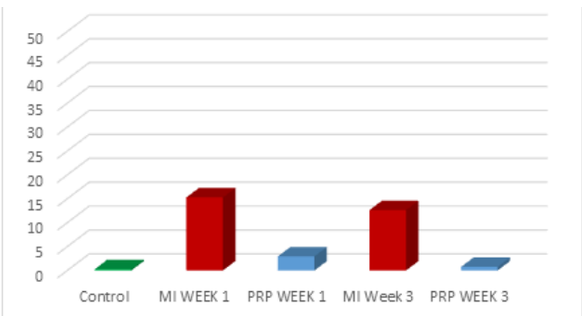

Chart 1: A bar chart showing mean area percentage of stained non-viable myocardial cells in different groups

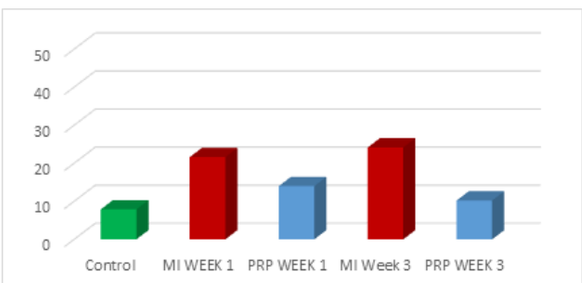

Chart 2: A bar chart showing mean area percentage of stained collagen fibers in different experimental groups

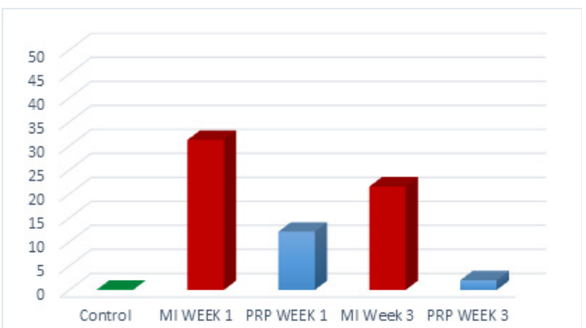

Chart 3: A bar chart Mean area percentage of immunoreactive cells for apoptosis between different groups

DISCUSSION

Myocardial infarction is a global liability of morbidities and mortalities. Moreover, it contributes to disability-adjusted life-years (DALYs) and greatly affects health-related quality of life (HRQoL)[27].

The purpose of the present study was to study the possible role of PRP in a model of myocardial infarction by isoproterenol in rat.

Experimental animals of choice were adult male albino rats as the injury produced by ISO is similar to myocardial injury in human. Authors defined young MI to be ≤ 45 years old[28]. Moreover, some authors reported increased incidence in men < 35 years[29].

Isoproterenol (ISO) was chosen for induction of myocardial injury in this work. It is a sympathomimetic drug acts on beta-adrenergic receptors and provides a practical, simple, and reliable induction model with lower mortality rate[17]. Subcutaneous route was chosen for injection of ISO as literature data stated that this simple route of administration significantly increases serological markers of myocardial injury, such as CPK, SGOT, CK-MB, SGPT, LDH and troponin[30].

PRP was of choice as a proposed healing promoting agent owed to its autologous non-chemical nature, no risk of host reaction, easily prepared and economically feasible[31].

Acute myocardial injury was evident in experimental animals after 12 hours form ISO administration. Myocardial fibers showed degeneration, increased sarcoplasmic eosinophilia and pyknotic nuclei. Separation and vacuolation of myocardial fibers could be explained by oedema in interstitium of myocardium[32]. It was suggested that pyknotic nuclei are the results of apoptosis[33]. Myocardial interstitium showed mononuclear cellular infiltration and vascular congestion with blood extravasated in myocardial tissue. Leucocytic infiltration was owed to release of ROS, leukotrienes, and hydrolytic enzymes[34]. Moreover, coagulative necrosis causes microvascular

injury and was found related to appearance of hemorrhagic areas in the myocardium[35]. Similar histological findings were reported by De Sánchez *et al*. (2012)[19].

After one week (pro-inflammatory phase), myocardial fibers showed an apparent increase in vacuolation, deeply homogenous acidophilic cytoplasm and pyknotic nuclei. Necrosis was confirmed by positive staining of cardiac cells with trypan blue vital stain as it enters cells with damaged cell membrane only. Apoptosis was confirmed by significant increase in caspase reactivity and brown staining of cytoplasm.

It has been illustrated that the process of apoptosis may not be complete in cardiac cells. Apoptosis occurred without nuclear fragmentation but with continuous loss of cytoplasmic proteins and contractile filaments. Lack of nuclear degradation make cells persist in the tissue but in a "zombie" state. Activation of β-adrenergic receptor by ISO induce apoptosis in cardiomyocytes of rat's ventricle by caspase-3 and ROS production which trigger mitochondrial permeability. These changes amplify amount of ROS produced and subsequently damage surrounding mitochondria causing eventually cellular death[36].

Loss of cross striations of cardiac fibers was confirmed by PTAH staining with presence of contraction band necrosis. Authors described contraction band necrosis as a sign of cell death that appears shortly after acute infarction which is characterized by an irreversible state of hypercontraction of cardiomyocytes and degradation of contractile apparatus of the cell. It is recognized by short sarcomeres and thick Z-lines. It was reported that experimental injection of catecholamines can produce cardiotoxicity through binding to β-adrenergic receptors and free radicals causing intracellular calcium overload that leads to cellular damage[37].

Cardiac interstitium showed after one week a significant increase in collagen deposition as detected by Masson's trichrome staining and an apparent increase in mononuclear cellular infiltration with noticed macrophages in the tissue. Macrophages are of two types: M1 which is related to pro-inflammatory response by releasing IL-6, IL-12, IL-1 and M2 that is concerned with anti-inflammatory response by releasing IL-10 for immunosuppression and induction of fibrosis by growth factors as well as matrix metalloproteinases[38].

After three weeks from ISO injection (healing phase), trypan blue positive cells as well as caspase positive cardiac cells showed non-significant decrease compared to one week ISO group. Masson's trichrome staining showed extensive collagen fibers replacing degenerated cells with apparent hypertrophy of healthy (non- affected fibers). Hypertrophy is an adaptive response to distribute increased wall stress more adequately with the presence of fibrous scar. Sarcomeric proteins, angiotensin-converting enzyme (ACE) and growth factors (insulin-like growth factor-1, transforming growth factor [TGF]-b1 & endothelin-1) are affected by hypertrophic stimuli. Hypotension produced by infarction causes an increase in secretion of natriuretic peptides, activation of RAS-aldosterone axis, and increase in catecholamine production. Also, local ACE activity is responsible for hypertrophy in non-infarcted myocardium[39].

Regarding PRP treated groups, cardiac fibers regained their branching and anastomosing architecture with restoration of cross striations in many regions. This was more evident after 3 weeks of treatment and was confirmed by PTAH staining of myocardium. Cardiac cellular death, as detected by immunohistochemical study of caspase 3 and trypan blue vital staining, showed significant decrease in one-week PRP-treated group and highly significant decrease in three weeks in comparison to ISO only treated groups. This finding suggests the possible role of PRP in limiting size of damaged myocardial tissue.

Crisci *et al*. (2018) reported storage and release of anti-apoptotic agents from PRP such as serotonin, HGF, sphingosine-1 phosphate and adenosine diphosphate. They also studied the effect of PRP microparticles in phosphorylation of AKT (a serine/ threonine protein kinase) improving endothelial survival[40]. Also, Angoulvant *et al*. (2011) referred anti-apoptotic effect of PRP to its role in reducing ROS and releasing IGF-1 that contributes in mitogenesis, differentiation, and inhibition of apoptosis in many organs, including the heart[41]. Fukaya *et al*. (2012) suggested the anti-apoptotic effect of PRP using caspase 3, LDH, and cell proliferation assay[42]. Also, Moussa *et al*. (2017) studied apoptosis related gene expression and reported a significant decrease in caspase & BAD genes and increased BCL-2 expression in PRP-treated groups suggesting the possible benefits of PRP with or without addition of stem cells[43].

Cardiac interstitium showed in PRP-treated groups after one week an apparent decrease in mononuclear infiltration which was markedly diminished after three weeks of treatment in comparison to ISO treated groups. The role of PRP in opposing inflammatory phase was not quite clear. Some authors referred it to enhancing mitochondrial depolarization and reduction of ROS by Catalase and superoxide dismutase enzymes[31]. Others attributed this to the anti-inflammatory cytokine Hepatocyte growth factor (HGF) released from PRP[44]. The concluded proposal from the present study is that PRP accelerates starting of healing phase, subsequently opposes the progression of inflammatory phase and this proposal was also supported by other researchers[40].

The present study showed also an improvement in blood vessels more that ISO only treated groups. Suryawan *et al*. (2022) supported these findings by cardiomyocytes differentiation assay and reported marked improvement with PRP administration[45]. This could be owed to the release of multiple growth factors from PRP such as transforming growth factor B (TGF-B1), platelet-derived growth factor BB (PDGF-BB), Insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and

epidermal growth factor (EGF). These factors enhance angiogenesis to rejuvenate damaged myocardium and attract bone marrow stem cells to be differentiated into endothelial cells and smooth muscle cells[46].

Collagen deposition in cardiac intersitium as detected by Masson's trichrome staining showed in PRP treated groups non-significant decrease after one week compared to ISO only treated group but with higher magnification, collagen fibers were seen in a two forms; replacement collagen of degenerated cardiac fibers and interstitial collagen between cardiac cells of fine regular type. Moreover, after one week of injury, recruitment of fibroblasts was noticed by light microscopy. These findings suggest the possible role of PRP in accelerating the beginning of healing process. On the other hand, after three weeks, PRP treated groups showed statistically significant decrease in collagen deposition compared to ISO only treated group. By higher magnification, collagen fibers were seen finely arranged between cardiac cells. This suggests the possible role of PRP in decreasing scar size and attenuation of adverse remodeling of the heart. Li *et al*. (2008) supported this result and stated that PRP accelerates collagen deposition in injured areas without similar effect on non-injured zones. They reported increase in immature collagen in one week of injury but decreased after 28 days with a significant increase in mature collagen in PRP-treated hearts[47].

Cardiac fibroblast stimulation can be owed to TGF-β that contributes to synthesis of collagen, expression of contractile genes specially, Fibroblast growth factor (FGF) and (VEGF) and also proteoglycans[48]. Amount and organization of collagen in the injured tissue are the two significant elements in restoration of tensile strength and opposing advancement of left ventricular dilatation after myocardial damage[49]. Following the deposition of collagen, the newly formed tissue matures by gradually losing ground substance and water and changing the collagen from type III to type I mature collagen fibers[44]. Authors studied the PDGF-α and PDGFR-β mediated pathways and reported their role in promoting collagen deposition in the injured tissue, decreasing unfavorable collagen deposition, formation of smaller mature scar without affection of vascular maturation[50].

CONCLUSION

PRP was found to accelerate starting of healing process by its anti-apoptotic and angiogenetic properties. This study found decreased collagen deposition, decreased necrosis and apoptosis, and restoration of branching cardiac architecture in PRP treated samples.

FURTHER RECOMMENDATIONS

Despite promising outcomes observed in the present work, further investigations with larger sample size and trials on human subjects are recommended. We suggest in further studies repeated injections of PRP and study exosome derived from platelets and its role in propagation of healing signals for longer distance.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

البلازما الغنية بالصفائح الدموية تخفف من الاصابة المستحثة بالأيزوبروتيرينول بعضلة القلب في ذكر الجرذان البيضاء البالغة: دراسة نسيجية و كيميائية مناعية

مروة محمد سليمان، مها مصطفي احمد زكريا، هاني شوقي نديم، ولاء عاطف حسن الفخراني، مريان مختار شكري، احمد سمير صادق

قسم التشريح وعلم الاجنة، كلية الطب، جامعة عين شمس، مصر

المقدمة: يعد احتشاء عضلة القلب عبئا عالميا خطيرا و الذي يؤثر بشكل كبير علي متوسط العمر و جودة الحياة. البلازما الغنية بالصفائح الدموية هي مصدر ذاتي لعوامل النمو و التي تتصف بخصائصها الشفائية.

الهدف من البحث: دراسة الدور المحتمل للبلازما الغنية بالصفائح الدموية في الحفاظ علي أنسجة القلب و تحسين عملية الشفاء في الاصابة المستحثة بالأيزوبروتيرينول بعضلة القلب.

المواد و طرق البحث: تم تقسيم ثمانية واربعين من ذكور الجرذان البيضاء البالغة الي ثلاثة مجموعات تضمنت المجموعة الضابطة (ثمانية عشر جرذ)، مجموعة الاصابة المستحثة لعضلة القلب (ثمانية عشر جرذ) و المجموعة المصابة و المعالجة بالبلازما الغنية بالصفائح الدموية (اثناعشر جرذا) . تم حقن عقار الأيزوبروتيرينول مرة واحدة تحت الجلد (٦٧ مجم / كجم) بينما تم حقن البلازما داخل القلب (١٠٠ميكرولتر) بعد مرور ١٢ ساعة من حقن الأيزوبروتيرينول. تم تجميع عينات من البطين الايسرللقلب بعد مرور ٧ ايام و بعد ٢١ يوما للدراسة النسيجية و الكيميائية المناعية.

النتائج: اظهرت الدراسة بالمجموعات المتلقاه عقار الأيزوبروتيرينول نخرا و موتا مبرمجا بخلايا القلب بالاضافة الي اختفاء التصدعات بنسيج القلب. كما اظهر النسيج الخلالي القلبي انتشارا لخلايا الالتهاب احادية الانوية و زيادة ذو دلالة احصائية بنسبة ترسيب الياف الكولاجين. و قد اظهرت العينات ايضا احتقانا بالاوعية الدموية مع نزف ملحوظ. بفحص المجموعات المعالجة بالبلازما الغنية بالصفائح الدموية؛ اوضحت العينات استعادة للبنية النسيجية لالياف عضلة القلب بالاضافة الي تقليل ذو مدلول احصائي للنخر و الموت المبرمج لخلايا القلب. كما اوضح النسيج الخلالي القلبي بهذه المجموعات انخفاضا ملحوظا بخلايا الالتهاب احادية الانوية و النزف. كان التباين ملحوظا بعد اسبوع و بعد ثلاثة اسابيع من تلقي البلازما و لكنه كان اكثر وضوحا بعد ثلاثة اسابيع. بينما كان الانخفاض بنسبة ترسيب الياف الكولاجين بأنسجة القلب ذو دلالة عالية احصائيا فقط بعد ثلاثة اسابيع بالمقارنة بمثيلاتها المتلقاه الأيزوبروتيرينول فقط.

الخلاصة: اظهرت البلازما الغنية بالصفائح الدموية القابلية علي تقليل موت الياف عضلة القلب كما انها تساهم في تسريع عملية الشفاء. استخدام البلازما الغنية بالصفائح الدموية قد يكون علاجا فعالا لاصابة عضلة القلب فهي بسيطة في التحضير ، ذات تكلفة مناسبة و بلا اضرار جانبية تذكر.