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Full Length Research Paper

Histological Study on the Protective Effect of Stem Cells and ginger on Isoproterenol Induced Myocardial Infarction in Male Albino Rats

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Till now no effective therapy for myocardial infarction is available, each of ginger extract and stem cell therapy were reported to be beneficial in ameliorating pathological progress of myocardial infarction. In this work we compared each of them and their combination on myocardial infarction. Forty male albino rats were divided into control group and Isoproterenol induced myocardial infarction group that was further subdivided into untreated infarction group, ginger treated group, Mesenchymal Stem Cells treated group and combined ginger with Mesenchymal Stem Cells treated group. Immunohistochemical staining for laminin and cardiac tropomyosin were applied on cardiac histological sections. Co-injection of Mesenchymal Stem Cells and ginger has a better protective effect on myocardial infarction in rats than either one alone as the heart appeared to be near normal condition as regard histological, immunohistochemical with laminin and cardiac tropomyosin, while there was moderate protective effect of Mesenchymal Stem Cells or ginger when used alone. Conclusions: This mode of protection, if proved to be effective in human, may provide a perfect way to decrease the high rate of mortality and morbidity due to coronary insufficiency and myocardial infarction.

Keywords: Mesenchymal Stem Cells, ginger, laminin, tropomyosin, myocardial infarction, Isoproterenol.

INTRODUCTION

Cardiovascular diseases (CVDs) have very high morbidity and mortality rates especially coronary heart diseases and myocardial infarction (Koene et al., 2016). Myocardial infarction is usually followed by many histopathological and biochemical alterations including hyperlipidemia, and elevated amount of free radicals (Ellulu et al., 2016). In most patients, pharmacological therapies as angiotensin converting enzyme inhibitors, adrenergic receptor blockers, and digitoxin may slow heart failure development in mild cases; however, severe cases may require continuous inotropic infusion, or even cardiac transplantation (Toma and Starling, 2010).

However, compatible donors, immune suppression, high cost and high mortality rates limit this procedure application.

Isoproterenol (ISO), is a b-adrenergic agonist, if administered in large doses in rats can produce severe myocardial stress leading to MI like injury as regard histological and physiological changes, so it was considered as a standard model for inducing myocardial infarction and studying the effects of many drugs and natural herbal products used in its treatment (Ojha et al., 2013).

Ginger is a commonly used spice with many health

benefits and was previously investigated for its many pharmacological effects as an anti-inflammatory agent, analgesic, chemoprotective, and antioxidant. Its antioxidant effects can protect against free radical damage that may contribute in pathogenesis of many cardiovascular diseases (Srinivasan, 2014). The association between antioxidant level and oxidative stress after myocardial infarction raised the possibility that high antioxidant levels may protect the heart by inhibiting LDL oxidation while low levels may harm the heart (Gunathilake and Rupasinghe, 2014).

Cardiomyocyte renewal may occur throughout life with a very slow rate and mild effect, due to endogenous cardiac stem cell activity or migration of stem cells from distant tissues. This repair after MI is inadequate to compensate for the severe loss of cardiomyocytes (Bei et al., 2016).

Human umbilical cord blood cells (HUCBCs) are rich in mesenchymal progenitor cells and contain a large number of endothelial cell precursors, as well as many stem cells with extensive in vitro proliferation capacity, so cell transplantation is considered as a promising therapeutic approach for MI in both laboratory studies and some clinical trials (Gong et al., 2016).

The aim of the present study is to compare the protective effects of ginger pretreatment versus mesenchymal stem cells (MSCs) treatment or the combination of both ginger and stem cells on ISO induced myocardial infarction in rats. Histological and immunohistochemical technique, morphometric measurements and statistical analysis of the data were used in this study.

MATERIAL AND METHODS

The original research was approved by the ethics committee in the Histology Department Faculty of Medicine Cairo University Egypt following international ethics and regulations for animal research in laboratory applications and all procedures were held under it (John et al., 2002).

Materials

Isoproterenol powder was purchased from Sigma Aldrich, St. Louis, Missouri, USA For Induction of myocardial infarction, the animals were injected subcutaneously with 85 mg/kg Isoproterenol daily for two successive days (Sun et al., 2015).

Ginger extracts were obtained from Arab Company for Pharmaceuticals and Medicinal plants MEPACO-Egypt, in tablet form, each tablet contained 400 mg Ginger extract.

In the present study, we used a dose 400 mg/ kg/ day by esophageal tube. The tablet was crushed and dissolved in 4 ml phosphate buffered saline (PBS).

Stem cells were obtained from human umbilical cord

blood and prepared according to Lu et al. (2010).

Fresh HUCB was collected immediately after delivery from the Department of Obstetrics and Gynecology, using heparinized 50 ml syringes then prepared in clinical pathology department faculty of medicine Cairo University.

Blood was slowly layered over Ficol Hypaque (Sigma, USA) at a ratio of 2:1 in sterile conical centrifuge tubes then centrifuged at 1200 rpm for 30 min at room temperature.

After centrifugation, the upper layer (plasma) up to 0.5 ml of the opaque interface containing mononuclear cells was carefully aspirated and discarded. The opaque mononuclear cell layer containing MSCs and HSCs (hematopoietic stem cells) was carefully aspirated and transferred to another sterile centrifuge tube.

A volume of 10 ml phosphate buffer solution (PBS) was added and mixed and centrifuged at 1200 rpm for 10 min to pellet the cells. The supernatant was then aspirated and discarded. This step was repeated twice.

An aliquot of separated cells was analyzed by flow cytometry to confirm the simultaneous expression of CD34 (HSCs hematopoietic stem cells markers) and CD44 (MSCs marker) within these cells.

Animals

The present study included 40 male adult albino rats weighing (200±10 gm). They were obtained from and housed in the animal house of Kasr- El-Aini Faculty of Medicine Cairo University. The animals received a standard diet for rodents and allowed free access to water. They were divided into 2 main groups each was further subdivided into 4 subgroups. Each subgroup was kept in separate wire cage at room temperature.

Animals were divided into two main groups:

Group I control group

Sixteen rats; to serve as control group: subdivided into four subgroups four rats each:

Group (I A): received 1 ml distilled water orally by oral gavage for 28 days then injected subcutaneously with 1 ml distilled water for two successive days at days 29 and 30 then injected with 1 ml PBS in the tail vein at day 31.

Group (I B): received ginger in a dose of 400 mg/ kg/ day dissolved in 1 ml distilled water orally by oral gavage for 28 days then injected subcutaneously with 1 ml distilled water for two successive days at days 29 and 30 then injected with 1 ml PBS in the tail vein at day 31.

Group (I C): received 1 ml distilled water orally by oral gavage for 28 days then injected subcutaneously with 1 ml distilled water for two successive days at days 29 and 30 then injected with 1000.000 mesenchymal stem cells suspended in 1 ml PBS in the tail vein at day 31.

Group (I D): received ginger in a dose of 400 mg/ kg/ day dissolved in 1 ml distilled water orally by oral gavage

for 28 days then injected subcutaneously with 1 ml distilled water for two successive days at days 29 and 30 then injected with 1000.000 mesenchymal stem cells suspended in 1 ml PBS in the tail vein at day 31.

Group II (MI induced group)

Twenty four rats; subdivided into four subgroups six rats each:

Group (II A) (ISO group): received 1 ml distilled water orally by oral gavage for 28 days then injected subcutaneously with 85 mg/kg of Isoproterenol dissolved in 1 ml distilled water for two successive days at days 29 and 30 then injected with 1 ml PBS in the tail vein at day 31.

Group (II B) (ISO-Ginger group): received ginger in a dose of 400 mg/ kg/ day dissolved in 1 ml distilled water orally by oral gavage for 28 days then injected subcutaneously with 85 mg/kg of Isoproterenol dissolved in 1 ml distilled water for two successive days at days 29 and 30 then injected with 1 ml PBS in the tail vein at day 31.

Group (II C) (ISO-stem cells group): received 1 ml distilled water orally by oral gavage for 28 days then injected subcutaneously with 85 mg/kg of Isoproterenol dissolved in 1 ml distilled water for two successive days at days 29 and 30 then injected with 1000.000 mesenchymal stem cells suspended in 1 ml PBS in the tail vein at day 31.

Group (II D) (ISO-Ginger-stem cells group): received ginger in a dose of 400 mg/ kg/ day dissolved in 1 ml distilled water orally by oral gavage for 28 days then injected subcutaneously with 85 mg/kg of Isoproterenol dissolved in 1 ml distilled water for two successive days at days 29 and 30 then injected with 1000.000 mesenchymal stem cells suspended in 1 ml PBS in the tail vein at day 31.

The rats were sacrificed at day 33 from starting the experiment which is three days from induction of myocardial infarction.

Rats were sacrificed after being anaesthetized with intraperitoneal injection 50 mg/kg thiopental sodium. Blood samples were collected for biochemical assays then the hearts were removed and the apical regions from the left ventricle fixed in 10% formol saline for 24 hours. Paraffin blocks were prepared and 5µm thick sections were stained with Haematoxylin and Eosin stain and immunohistochemical staining technique for laminin and tropomyosin (Peter and David, 2013).

Biochemical assays: The serum levels of cardiac creatine kinase-MB (CK-MB) were determined by taking blood sample from the tail vein prior to sacrificing the animals and measured using Isoenzyme ELISA Kit (Catalog #MBS008782). (MyBioSource company, San Diego, California, USA).

Quantitative morphometric analysis and statistical analysis of the obtained chemical and immunohistoche-

mical results were done (Emsley et al., 2010).

Mortality rate

Three rats from group II A and one rat from each of groups II B, II C, II D died before the end of experiment.

Immunohistochemical staining

Sections from all animals were processed simultaneously. Tissue samples were fixed in 10% formalin, processed and embedded in paraffin wax blocks. Sections were cut at 5 micron thickness and mounted on poly-L-lysine-coated microscope slides. Sections were labeled with an indirect streptavidin–biotin–peroxidase immunohistochemical staining for the presence of laminin using (ready-to-use Ab-1, rabbit polyclonal antibody Cat. #RB-082-R7, NeoMarkers, Fremont, CA 94538, USA) and Tropomyosin Ab-1 using ready-to-use Mouse Monoclonal Antibody Cat. #MS-1256-R7, NeoMarkers, Fremont, CA 94538, USA) The sections were deparaffinized and rehydrated by passing through two changes of xylol, two changes of 100%, then 90% and 70% ethyl alcohol then two changes of distilled sterile water, for 15 min each. Sections were treated with 3% H₂O₂ and methanol for 30 min to block endogenous peroxidase activity then washed in phosphate- buffered saline (PBS 3 changes, 2 minutes each). Excess buffer was drained and non-specific background was eliminated by covering the sections immediately with 2 drops of serum blocking solution and incubated for 10 minutes at room temperature then excess serum was eliminated. Primary antibody was applied to the sections, 2 drops for each section and incubated in humidity chamber for 60 minutes at room temperature. Sections were washed with PBS (3 changes 2 minutes each), then incubated with biotinylated polyvalent secondary antibody Histostain SP kit Cat. #95-9643 (LAB-SA system, Zymed Laboratories Inc, San Francisco, CA 94080, USA,) for 20 min. Sections were rinsed well with PBS and incubated with "Streptavidin-Horseradish peroxidase" 2 drops for each section) then incubated for 10 minutes in the humidity chamber. Slides were washed well in PBS (3 changes, 2 minutes each) and then dried around the edges of the sections using a piece of gauze. Substrate-chromogen mixture was prepared (Reagents 3A, 3B, 3C) by adding one drop of concentrated substrate buffer, one drop of concentrated DAB chromogen and 1 ml of 0.6% hydrogen peroxide to one ml of distilled water. The mixture was mixed well and protected from light and applied immediately to the sections (2 drops for each section). Slides were incubated at room temperature for 5-10 minutes then Slides were rinsed well with distilled water and they were counterstained with hematoxylin. Slides were washed in tap water until blue. Slides were put in PBS, and then rinsed in distilled water. The slides were dehydrated and mounted.

Negative controls for all groups were performed by replacing the primary antisera with PBS.

Laminin or tropomyosin positive cells showed brown cytoplasmic deposits.

Positive control was a specimen of kidney for laminin and a specimen of skeletal muscle for tropomyosin.

Quantitative Morphometric Analysis

The following parameters were examined using the Leica Qwin 500 Image analysis: For each parameter; measurement was done in five randomly selected non overlapping fields per slide from five slides of each animal. The magnification used for all parameters was $\times 400$.

Mean laminin immunostained area percent.

Mean tropomyosin immunostained area percent

Statistical analysis methods

Statistical analysis was performed on "EXCELL" Statistical Analysis System Software. Data were presented as the Mean \pm SD. Differences among the study groups were detected by one way analysis of variance (ANOVA) as global test to determine any differences in data prior to comparing pairs of groups then "student-t test" was performed. P values < 0.05 were considered statistically significant (Jennifer and Belinda, 2005).

RESULTS

Histological Results

Histopathological observations on cardiac tissues of control rats showed clear cell membrane integrity, normal myofiber structure with irregular striations, branching of fibers and continuity with adjacent ones. The cytoplasm appeared acidophilic and nuclei were large and pale. Multiple capillaries appeared between the myofibers. There was no evidence of inflammatory cell infiltration or edema in all control subgroups. Weak membranous immunoreactivity for laminin in cardiomyocytes and basal lamina of blood vessels and strong cytoplasmic immunoreactivity for tropomyosin in most cardiomyocytes was observed. There was no remarkable histological differences among different control subgroups (Figure 1).

However, the tissues of ISO-induced rats showed deeply acidophilic cytoplasm, loss of striations and necrosis of myofibrils. Degenerated myofibrils were accompanied by loss of nuclei in some cells and infiltration of neutrophil and macrophages with appearance of interstitial edema and many congested capillaries between the fibers. Strong membranous immunoreactivity for laminin in cardiomyocytes and basal lamina of blood vessels and weak cytoplasmic immunore

activity for tropomyosin in most cardiomyocytes with even few negatively stained cells were observed (Figure 2).

Pre-treatment with ginger (400 mg/kg) or treatment with stem cells reduced the severity of myocardial necrosis with lesser degree of muscle separation and mild neutrophil infiltration and edema in representative tissues. The improvements were observed to be more obvious in mixed group with pre treatment with ginger and treatment with stem cells after myocardial infarction as the tissue was shown to have clear integrity, near to normal tissue histology, with slight evidence of focal necrosis and inflammatory cell infiltration in some animals while some other animals appeared with myocardial structure that was almost comparable with the control group. Moderate membranous immunoreactivity for laminin in cardiomyocytes and basal lamina of blood vessels and moderate cytoplasmic immunoreactivity for tropomyosin in most cardiomyocytes in rats pre-treated with ginger (400 mg/kg) or treated with stem cells while in combined group rats pretreated with ginger and treated also with stem cells there was mild membranous immunoreactivity for laminin in cardiomyocytes and basal lamina of blood vessels and strong cytoplasmic immunoreactivity for tropomyosin in most cardiomyocytes (Figures: 3, 4, 5).

Morphometric Results

ANOVA among groups as regard CK-MB level, mean area % of Laminin and Tropomyosin immunoreactivity showed that, in each parameter, there was statistically significant variance among different groups were $P < 0.05$.

So comparison between each two groups in these parameters was performed using t-test and the results were summarized in tables (1 and 2):

Table 1 summarize the CK-MB serum level:

CK-MB level revealed the highest value in group II A (untreated ISO induced myocardial infarction group) while the least one was in group I then group II D (control group then the group given both ginger and stem cells).

Table (2) summarize the immunohistochemical results:

Mean area % of Laminin immunoreactivity revealed the highest value in group II A (untreated ISO induced myocardial infarction group) while the least one was in group I and group II D (control group and the group given both ginger and stem cells).

The least value of Area % of Tropomyosin was detected in group II A (untreated ISO induced myocardial infarction group). While the greatest one was in group I and group II D (control group and the group given both ginger and stem cells).

Comparing each two groups together; it was found to be statistically significant (P value < 0.05) except between the following; where no statistical significance in both parameters could be observed:

*Group I and group II D

*Group II B and group II C

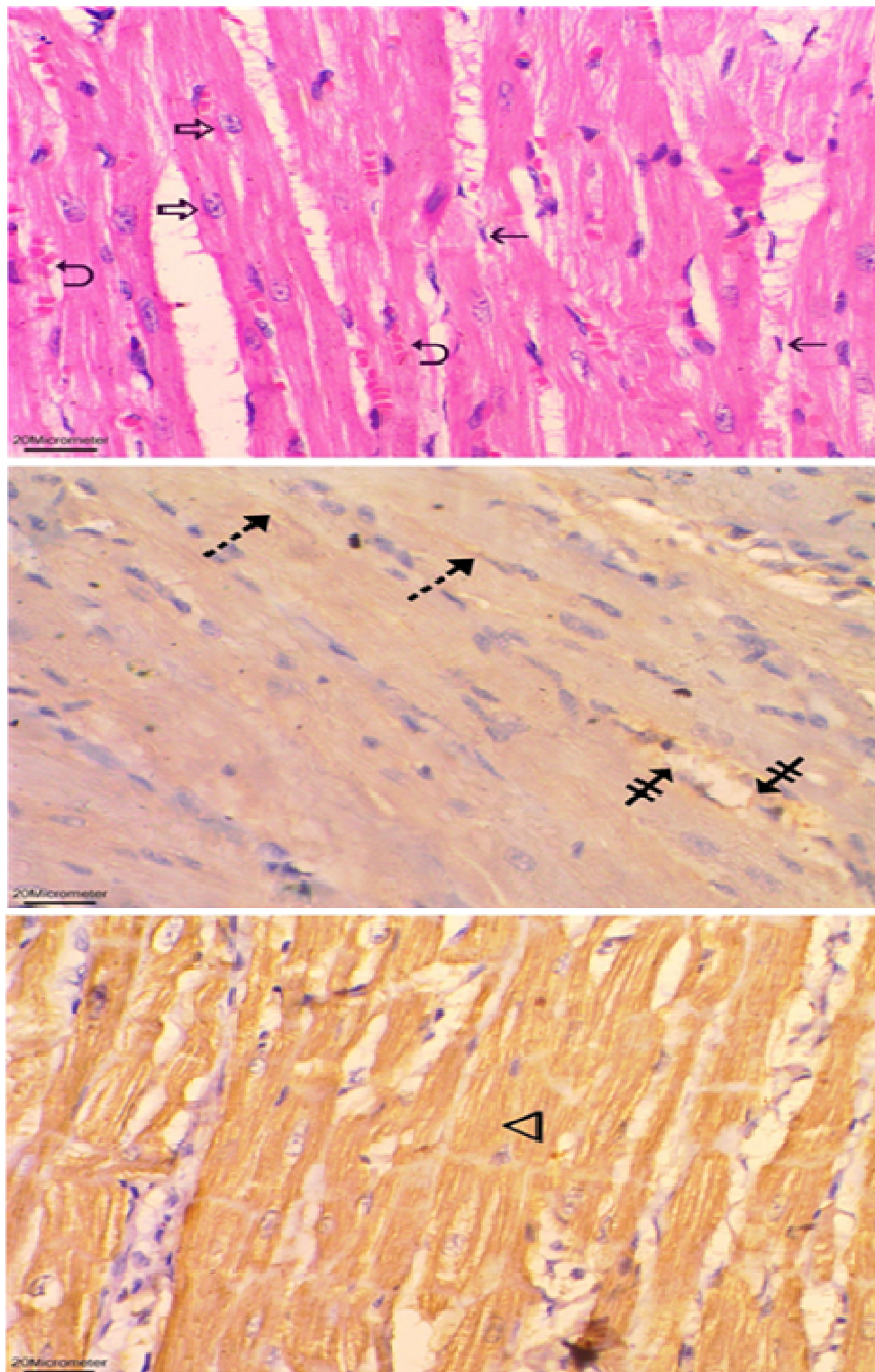


Figure 1. control group:
Photomicrographs of sections of heart from group I (control group) showing cardiac muscle fibers with cross striations and centrally located nuclei (thick arrows). Each fiber is surrounded by endomysium containing fibrocytes (thin arrows) with flattened dark nuclei, and many blood capillaries (curved arrows) are running alongside the muscle fibers. There is mild membranous immunoreactivity for laminin in cardiomyocytes (dotted arrow) and in basal lamina of blood capillaries (crossed arrow) and strong cytoplasmic immunoreactivity for tropomyosin in cardiomyocytes (arrow heads).
[(a) H&E stain (b); Laminin immunostaining; (c) Tropomyosin immunostaining] x400; scale bar 20 micrometer.

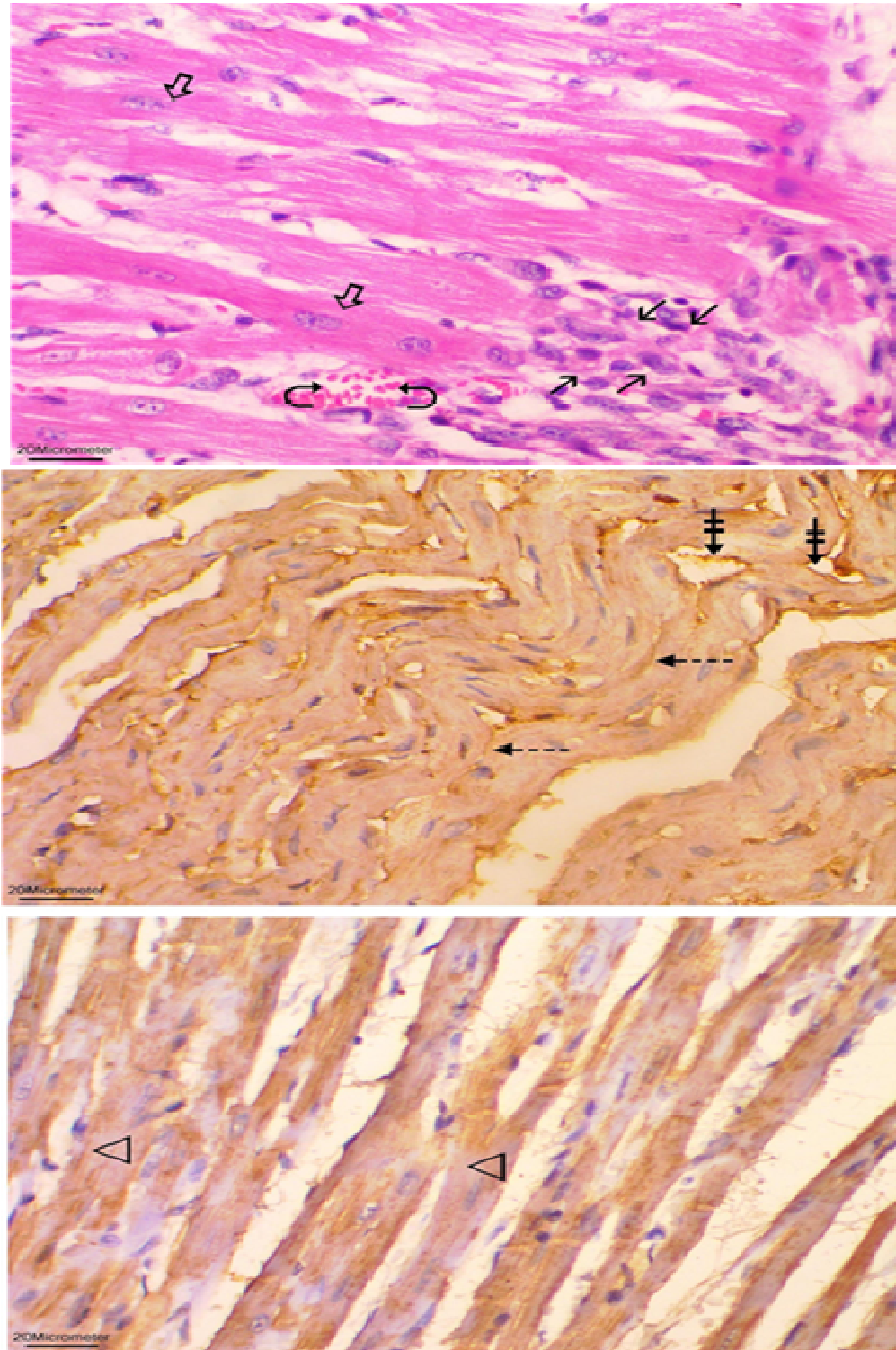


Figure 2. Untreated myocardial infarction group: photomicrographs of sections of heart from group II A (ISO induced myocardial infarction without treatment) showing interrupted and separated deeply acidophilic cardiac muscle fibers (thick arrows) with less striations of fibers. There are markedly dilated congested blood capillaries (curved arrows) and dense mononuclear cell infiltration (thin arrows) in between the muscle fibers. There is very strong membranous immunoreactivity for laminin in cardiomyocytes (dotted arrow) and in basal lamina of blood capillaries (crossed arrow) and weak cytoplasmic immunoreactivity for tropomyosin in most cardiomyocytes (arrow heads). [(a) H&E stain (b); Laminin immunostaining; (c) Tropomyosin immunostaining] x400; scale bar 20 micrometer.

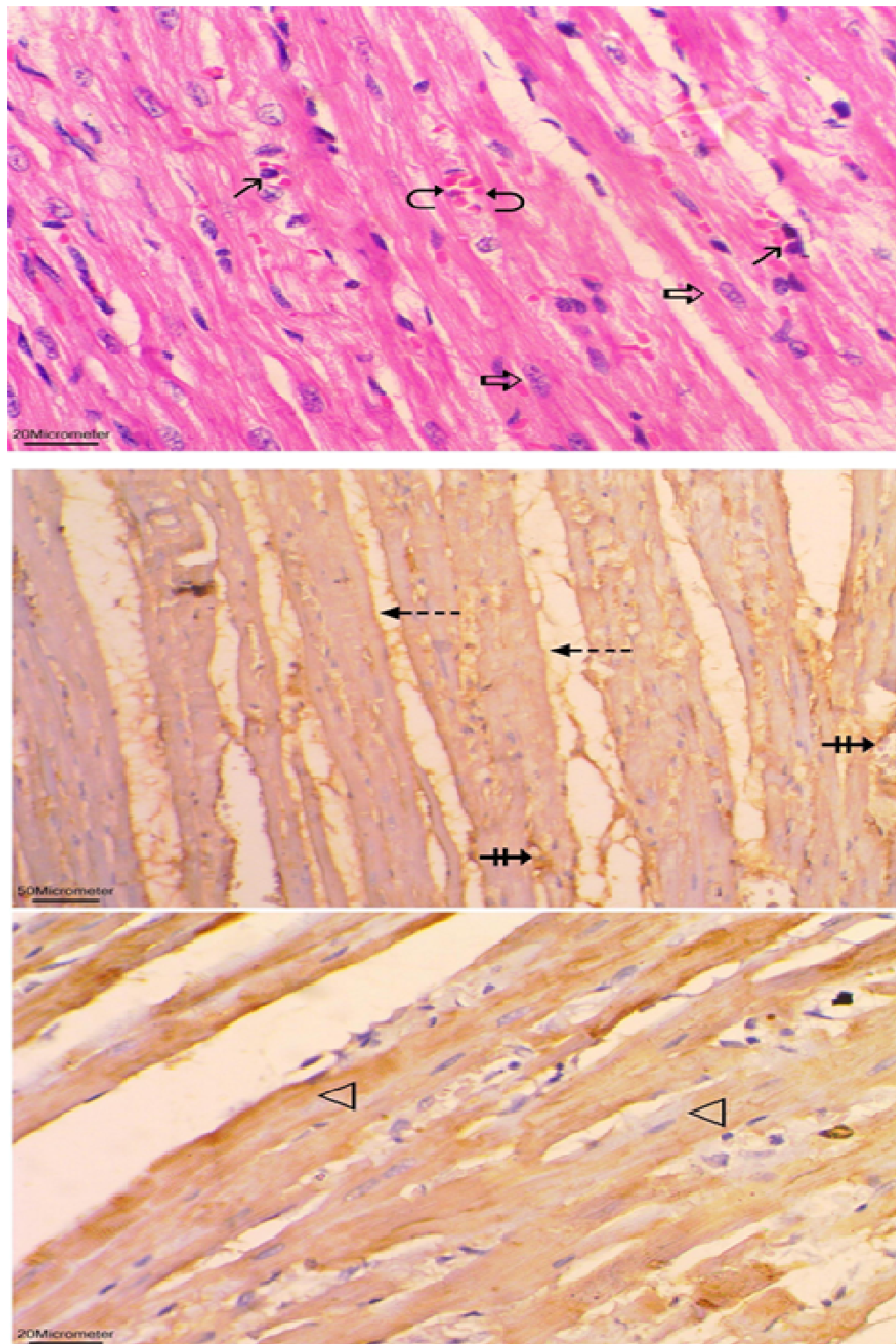


Figure 3. Ginger treated group: Photomicrographs of sections of rat heart from group II B (ISO induced myocardial infarction with ginger administration) showing normal arrangement of cardiac muscle fibers (thick arrows) with little interstitial spaces and mildly congested capillaries (curved arrows) and occasional cellular infiltration in between muscle fibers (thin arrows). There is moderate membranous immunoreactivity for laminin in cardiomyocytes (dotted arrow) and in basal lamina of blood capillaries (crossed arrow) and moderate cytoplasmic immunoreactivity for tropomyosin in cardiomyocytes (arrow heads). [(a) H&E stain (b); Laminin immunostaining; (c) Tropomyosin immunostaining] x400; scale bar 20 micrometer.

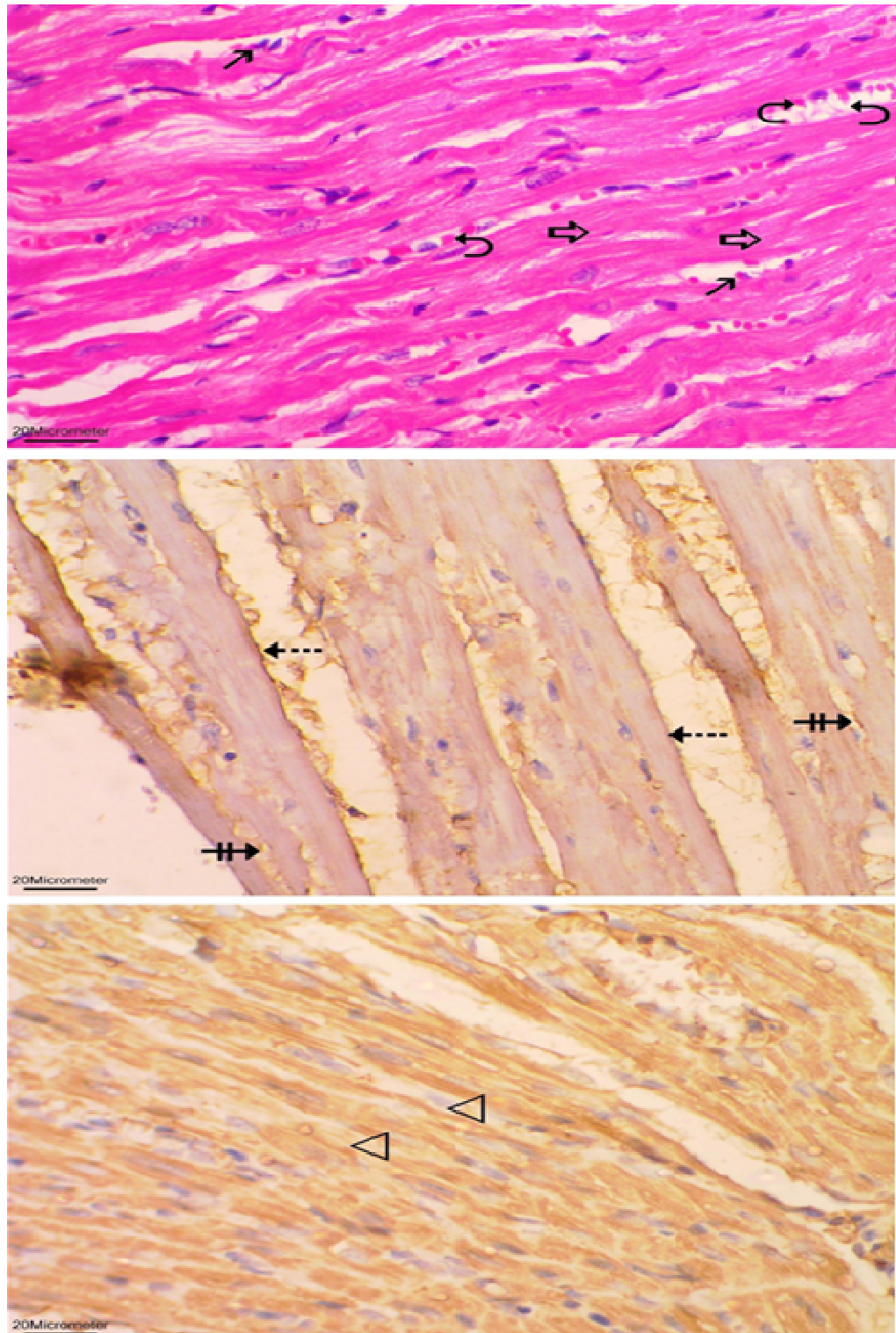


Figure 4. Stem cells treated group:

Photomicrographs of sections of rat heart from group II C (ISO induced myocardial infarction with stem cells administration) showing normal arrangement of cardiac muscle fibers (thick arrows) with little interstitial spaces and mildly congested capillaries (curved arrows) and occasional cellular infiltration in between muscle fibers (thin arrows). There is moderate membranous immunoreactivity for laminin in cardiomyocytes (dotted arrow) and in basal lamina of blood capillaries (crossed arrow) and moderate cytoplasmic immunoreactivity for tropomyosin in cardiomyocytes (arrow heads).

[(a) H&E stain (b); Laminin immunostaining; (c) Tropomyosin immunostaining] x400; scale bar 20 micrometer.

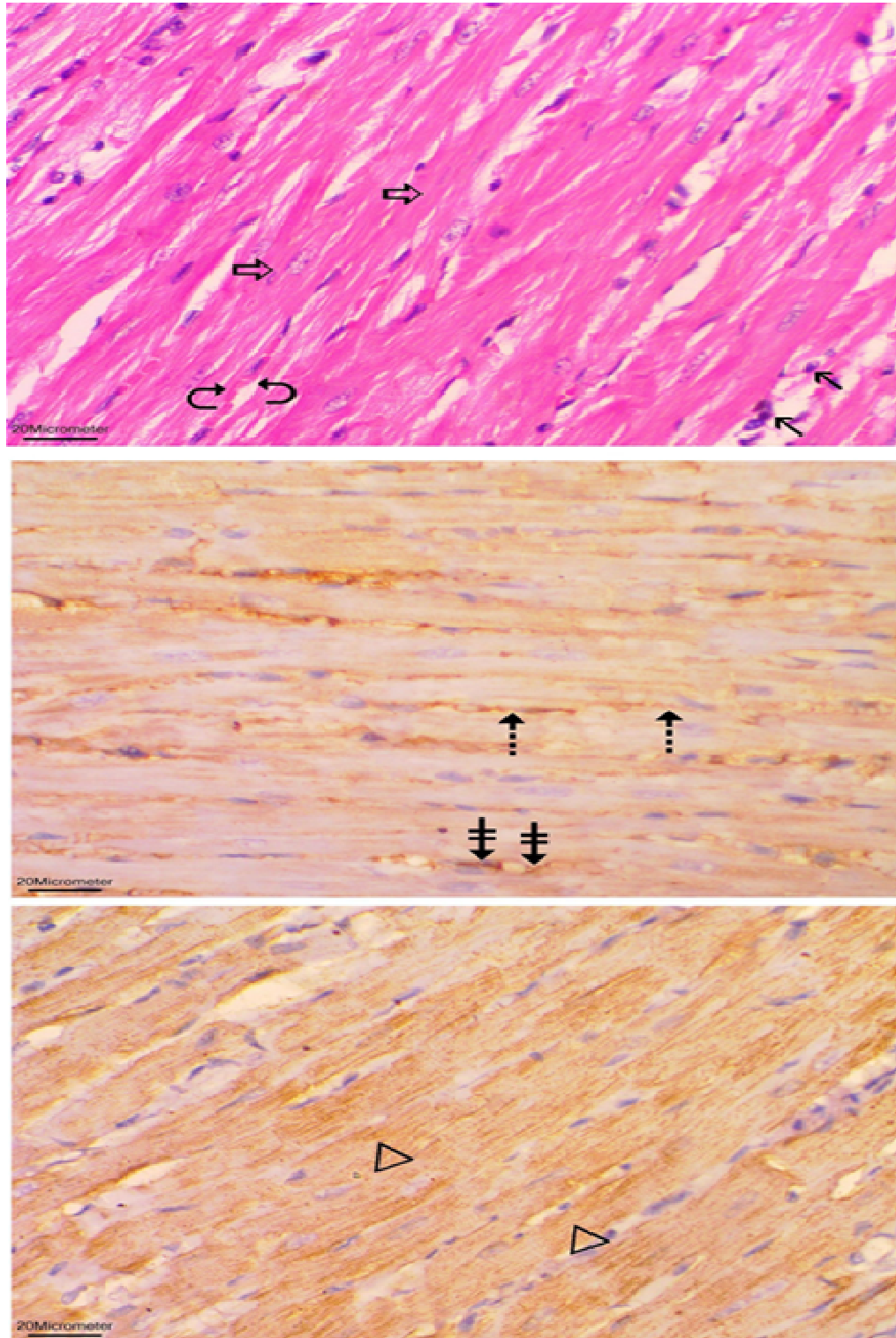


Figure 5. Ginger and stem cells treated group:
Photomicrograph of sections of rat heart from group II D (ISO induced myocardial infarction with ginger and stem cells administration) showing normal arrangement of cardiac muscle fibers (thick arrows) with minute interstitial spaces containing fibrocytes (thin arrows) without any apparent cellular infiltration and normally appeared capillaries in between muscle fibers (curved arrows). There is mild membranous immunoreactivity for laminin in cardiomyocytes (dotted arrow) and in basal lamina of blood capillaries (crossed arrow) and strong cytoplasmic immunoreactivity for tropomyosin in cardiomyocytes (arrow heads). [(a) H&E stain (b); Laminin immunostaining; (c) Tropomyosin immunostaining] x400; scale bar 20 micrometer.

Table 1. Mean CK-MB± STDEV

	Control	ISO	ISO-Ginger	ISO-Stem cells	ISO- Ginger- Stem cells
Mean CK-MB ± STDEV	71±10.45 ^a	183.615±11.55 ^b	127.846±15.609 ^c	126.615±16.103 ^c	104.154±13.44 ^c

ISO: Isoproterenol

Different superscripts indicate statistically significant difference (P < 0.05) compared to other groups.

Table 2. Immunohistochemical results of Laminin and tropomyosin (mean area %± STDEV):

	Control	ISO	ISO-Ginger	ISO-Stem cells	ISO- Ginger- Stem cells
Mean Laminin area %± STDEV	2.16077±0.67 ^a	5.17±1.46 ^b	3.70077±1.23 ^c	3.43385±0.85 ^c	2.76462±0.69 ^a
Mean tropomyosin area %± STDEV	36.0769±3.04 ^a	22.6154±2.89 ^b	27.1538±3.93 ^c	27.3846±4.04 ^c	32.3077±3.51 ^a

ISO: Isoproterenol

Different superscripts indicate statistically significant difference (P < 0.05) compared to other groups.

DISCUSSION

Myocardial infarction with loss of functional myocardium is the major etiology for heart failure. Even with rapid primary therapy, prognosis remains bad in patients with large infarction with left ventricular dysfunction. So, it would be highly desirable to influence healing of myocardial infarction to maintain structure and function of the heart (Ertl and Frantz, 2005).

This work aimed at investigating the role of ginger or intravenous injection of mesenchymal stem cells from umbilical cord blood either separate or in combination in the treatment of myocardial infarction induced by isoproterenol in male albino rats. Histological and immunohistochemical techniques in addition to biochemical markers analysis and morphometric measurements and statistical analysis of the data were applied in this study.

In the present study, myocardial infarction (MI) was induced by ISO in rats in a dose of 85 mg/kg for two successive days. Generation of highly cytotoxic free radicals was implicated as one important causative factor in ISO-induced myocardial damage (Xu et al., 2008). This auto-oxidation resulted in free radical-mediated peroxidation of membrane phospholipids leading to permeability changes in the myocardial membrane, intracellular calcium overload, and cellular damage observed in clinical conditions such as angina, transient myocardial hypoxia, acute coronary insufficiency (Adámková et al., 2011).

The ISO-induced myocardial infarction model is advantageous as less invasive procedures were applied avoiding the complications of general anesthesia and sutures remaining in coronary artery ligation model (Jiang et al., 2011).

The recommended doses of ginger with very high safety profile for human range from 1- 3 gm daily. Adverse effects of ginger in humans are uncommon but it may cause mild diarrhea. Ginger is on the U.S. Food and Drug Administration's GRAS (generally accepted as safe)

list. The British Herbal Compendium documents no adverse effect of ginger (Lakhan et al., 2015).

In the present study, we used a dose of ginger 400 mg/ kg/ day which is equivalent to 3 gm/ day for human average body weight 60 kg which is the highest safe recommended dose used in different indications (Reagan-Shaw et al., 2008).

The elevation of reactive oxygen species and/or decreasing of antioxidants lead to the formation of oxygen and hydrogen peroxide that is toxic and may cause oxidative stress and affect the pathogenesis of myocardial infarction (Burn and Varner, 2015).

This event was supported by previous reports mentioning the ability of ginger in maintaining normal levels of antioxidant enzymes and gave protection against oxidative tissue damage (Amran et al., 2015).

MSCs are considered as undifferentiated cells that are capable of self renewal and differentiation into several cell types. MSCs can be isolated from different sources as bone marrow, umbilical cord blood, adipose tissue, placenta etc. Umbilical cord blood is regarded as a major source of MSCs. Compared with other MSCs sources, hUCB-MSCs could be considered as a very good source for use in stem cell therapy (Heo et al., 2016).

Goyal et al. (2015) suggest that cardiac troponin I (cTnI) and CK-MB are sensitive and specific biomarkers in laboratory animals correlated with cardiotoxic drugs induced histological cardiac damage. They are not usually found in serum but are released due to necrosis, so they are important indicators in diagnosis of MI as they are highly sensitive and specific (Hashmi and Al-Salam, 2015). In the present study, we assessed CK-MB to assess the severity of MI. Increased activities of this marker enzyme in serum indicated cellular damage and elevated cell membrane permeability implicated in the pathogenesis of myocardial necrosis (Goyal et al., 2015). In addition to that the cell membrane may rupture, due to deficient oxygen supply or glucose, resulting in leakage of enzymes into the blood stream with higher serum concentration (Bao et al., 2012). In our study we found

that pretreatment with ginger extract (400 mg/kg) lowered the activity of CK-MB by preserving the structural and functional integrity and myocardial cell membrane permeability, restricting enzymes leakage. Previous studies reported inversed proportion relationship between antioxidants and the severity of oxidative stress as ISO-induced MI (Nagoor et al., 2016). However, all compounds used in the current study reduced levels of CK-MB and ameliorated histopathological cardiac damage to some extent, consistent with previous studies (Sahu et al., 2016).

The new finding of this study is that combination of ginger and stem cells had greater cardioprotective effect than either one alone. The effects of combination of ginger and stem cells in rats with ISO-induced myocardial injury have not been reported previously. Oxidative stress induced the formation of ROS, which played important roles in cardiac pathology. The harmful effects of ROS could be due to their direct interaction with cellular lipids, proteins, and DNA, causing cellular damage and death (Li et al., 2015). Our results showed that pre-treatment with ginger or treatment with stem cells or combination may protect against myocardial injury via antioxidative effects.

Histopathological examination on the myocardial tissue of the control group showed normal morphological cardiac structure without any signs of necrosis while myocardial tissue of ISO-induced MI rats were infiltrated with leukocytic cells and showed large areas of coagulative necrosis and pale myocytes with pyknotic nuclei and decreased striations in addition to separation of cardiac muscle fibers and diffuse interstitial edema.

Pretreatment with ginger showed a lesser degree of cardiac muscle fibers separation, necrosis, and inflammatory cell infiltration and when combined with stem cells, the myocardium showed near to normal morphological architecture that may confirm the cardioprotective effects of ginger and stem cells.

Up till now, there is no specific agent being used to control cardiac remodeling. Following the administration of ISO, there was a significant increase in the heart to body mass ratio may be due to irregular widening of the interstitial space and increased water content in the hypertrophied heart (Fraccarollo et al., 2014).

Similarly, Lobo Filho et al. (2011) found myofibril degeneration and necrosis in myocardial tissue 24 hours after Isoproterenol injection. They attributed this to leukocytosis which contributed to myocardial injury due to the release of leukotrienes, reactive oxygen species (ROS) and hydrolytic enzymes in addition to reduction of free radical scavenger enzymes such as catalase.

ISO administration increased the levels of nitrite; an oxidized end-product of nitric oxide that was subsequently reduced by ginger treatment. Restored levels of myocardial nitrite with ginger treatment may be attributed to its antioxidant activity (Ahmed, 2016).

Mononuclear cells infiltration observed in this study was

consistent with the findings of Haleagrahara et al. (2011) who explained that the molecular events during MI relate to the initial ischemic event, reperfusion, and subsequent inflammatory response. Up to 6 hours following the initial ischemic event, most cell loss occurs via apoptosis. After that, necrosis predominates. Ischemic endothelial cells express adhesion molecules that attract neutrophils towards damaged myocardium.

In myocardial infarction; the capillary network is unable to support the greater demands of the hypertrophied myocardium, resulting in progressive loss of viable tissue, infarct extension and fibrous replacement. It is possible that newly formed vessels (neovascularization) after MSCs transplantation improve tissue perfusion around the ischemic boundary zone, decreased apoptosis of hypertrophied myocytes in the peri-infarct region, survival of viable myocardium, reduction in collagen deposition and sustained improvement in cardiac function (Bai et al., 2013).

MSCs may contribute to neovascularization in the ischemic myocardium through growth factor-mediated paracrine regulation (Santos et al., 2014).

In the (MI) group sections of the rat heart with tropomyosin immunostaining showed strong immunoexpression in many cardiac muscle fibers, mild in some other fibers and negative in few fibers. These findings were confirmed by statistical analysis, which reflects gradual decrease in the percentage of healthy cardiac muscle when compared with control group.

As regard MSCs injected rats they showed immunoexpression of tropomyosin in many cardiac muscle fibers. This was in agreement with Henning et al. (2007) who reported that IV injection of 500 000 HUCBC produced significant reduction in infarct size without requirements for host immune suppression.

In our work, MSCs were used in the treatment of MI because their transplantation prevents the thinning of left ventricle wall, decreases infarct size and induces little interstitial mononuclear cell infiltration as reported also by Cambria et al. (2016). The superior ability of MSCs to migrate from the site of injection to the infarcted zone than HSCs transplantation could help them to exert their paracrine effect. In addition to that, hypoxia or inflammatory mediators in ischemic area could be responsible for MSCs migration (Trounson and McDonald, 2015).

In the group given both ginger and stem cells, sections of rat heart stained with tropomyosin immunostaining showed positive expression in many cardiac muscle fibers which was confirmed by statistical analysis. There was significant increase in the percentage of healthy cardiac muscle when compared with the group taking either ginger or stem cells only.

So we can conclude that ginger and stem cells may protect the heart to some extent from severe injury during the process of myocardial infarction but the combination of both may give superior results.

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