Original Article	**The Possible Enhancement Effect of Vitamin E on the Mesenchymal Stem Cell Treatment of Isoproterenol Induced Myocardial Infraction in Male Albino Rats**

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ABSTRACT

Background: Survivors of myocardial infarction develop scarring followed by ventricular remodeling despite optimum medical care . Stem-cell-based therapy has been given increased attention in terms of its potential contribution to cardiovascular regeneration . However, the therapeutic potential of MSCs is hindered by their low survival rate after transplantation in damaged myocardium.

The Aim of the Present Study: Is to find out whether vitamin E can enhance the efficacy of mesenchymal stem cell treatment of isoproterenol-induced myocardial Infarction in rats or not.

Material and Methods: Fifty Albino rats were divided into 5 equal groups :Group I (Control group): Rats received 1 ml of normal saline SC for 4 weeks. Group II (Isoproterenol group): Rats were given by SC injection 85 mg Isoproterenol / kg.b.w. once daily for two successive days. Group III (Vit E–isoproterenol "ISO" group): Rats were treated with ISO once daily for 2 days and after 1 week, they received Vit E(100mg/kg b.w./day) orally for 1 week. Group IV (Stem cell group): The rats were treated with ISO for 2 days as in group II, and after 1 week from the last dose of ISO, the animals had received MSCs intravenously with 2x1o6 cells/ rat . Group V (stem cell and vit. E group) : The rats were treated with ISO and Vit. E as in group III, and after the last dose of Vit.E, the animals were injected with the MSCs intravenously as in group IV. After rat scarification ,the sections of hearts were stained with Hematoxylin-Eosin (HE) and Masson's Trichrome stains .Also Immunohistochemical study was done to detect caspas-3 and CD105. Morphometric study: The mean area percentage of collagen fiber deposition and Caspase immuno-expression was quantified in five images from five non-overlapping fields of each rat . The data were collected from the experiment , recorded and analyzed using IBM SPSS Statistics software.

Results: In ISO group , there were a wide separation of cardiac muscle fibers with extravasation of blood. In ISP and vit. E group ,there were a moderate separation of cardiac muscle fibers and extravasation of blood. In ISO &stem cell group , there were slight separation of cardiac muscle with minimal extravasation In ISO ,vit.E and stem cell group ,the cardiac muscle fibers were nearly similar to control group but with minimal extravasation . In this group, there was a minimal amount of collagen fibers when compared with groups II ,III and IV . Also, it showed positive caspase -3 immune reaction . As to CD 105 ,this group also showed more positive cytoplasmic reaction in regenerating cardiac muscle.

Conclusion: We can conclude that if either Vit.E preparations or stem cells are given alone after myocardial infarction, some improvement of myocardial fibers occurs but when they are given together(VitE. And stem cells), better results are obtained

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Key Words: Acute myocardial infarction, caspas-3, isoproterenol, stem cells, vit. E.

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INTRODUCTION

Cardiovascular disease (CVD) is one of the main causes of death (From 1999 to 2009) . The rate of death due to CVD has declined, but nevertheless the burden of disease remains high ***(Go, et al. 2013)***. Although improved medical care and acute management of myocardial infarction have led to a considerable reduction of early mortality rate, survivors are susceptible to an increased prevalence of chronic heart failure as they develop scarring followed by ventricular remodeling despite optimum medical care ***(Jeevanantham et al.,2012)***. The main issue of current pharmacological, interventional or operative therapies is their disability to compensate the irreversible loss of functional cardiomyocytes ***(Steinhauser and Lee., 2011)*** Hence, the future challenge of cardiovascular therapies will be the functional regeneration of myocardial contractility by novel concepts, like cell based therapy, tissue engineering or reprogramming of scar fibroblasts ***(Assmus and Zeiher., 2013)***.

During the past decade, many clinical trials showed positive results of cell therapy ***(Makkar et al., 2012)*** ,while other clinical studies showed no beneficial effect of cell therapy over placebo ***(Sürder et al., 2013)***.

Stem-cell-based therapy has been given increased attention in terms of its potential contribution to cardiovascular regeneration. Previously published data showed that mesenchymal stem cells (MSCs) had been widely applied in regenerative medicine and exhibited beneficial effects on postinfarct hearts ***(Bartunek, et al., 2013)***. However, the therapeutic potential of MSCs is hindered by their low survival rate after transplantation in damaged myocardium. Therefore, how to enhance MSC survival under such a condition is a crucial problem to improve MSC mediated benefits in postinfarct hearts ***(Bartunek et al., 2013)***.

Isoproterenol, a beta-adrenoceptor agonist, has been reported to produce MI in large doses. Upon auto-oxidation, isoproterenol generates highly cytotoxic free radicals known to stimulate the peroxidation of membrane phospholipids causing severe damage to the myocardial membrane. Hence, it is widely used as a model to produce myocardial infarction in rats ***(Kannan and Quine., 2013)***.

Vitamin E (vit E) is the most widely used vitamin in food Supplements. Owing to its wide array of biological actions, public and scientific interests have been directed towards the role of vit E in health promotion and disease prevention ***(Mukesh et al., 2007)***. It is a predominant lipophilic antioxidant in plasma membrane and tissues and is the most abundant antioxidant in low-density lipoprotein (LDL). Beside having antioxidant properties, vit E has been shown to slow or inhibit the oxidative modification of LDL that is responsible for development and progression of atherosclerosis ***(Munteanu et al., 2004)***. Moreover, high levels of vit E have been measured in the mitochondria, Golgi apparatus, lysosomes, and endoplasmic reticulum ***(Saldeen et al.,1999)***.

The aim of the present study is to evaluate the efficacy of vitamin E in mesenchymal stem cell treatment of isoproterenol-induced myocardial Infarction in rats.

MATERIALS AND METHODS

I- Materials

1- Isoproterenol (ISO) hydrochloride was purchased in the form of a white powder from Sigma Chemical Company. It was administered subcutaneously daily at a dose of 85 mg/kg b.w. dissolved in 5 ml of normal saline (0.9% NaCl) for 2 days ***(Mehdizadeh et al., 2013)***.

2- Vitamin E is available commercially as E–Viton capsules produced by Kahira Pharm. and Chem.Ind. Company. Each capsule contains 100 mg α-tocopherol acetate. The recommended dose of vitamin E in rats is 100mg/kg/day. The content of one capsule was dissolved in 30ml corn oil ***(Aman and Ramachandran, 2009)***.

3- Isolation, culture and labeling of MSCs from rat bone marrow *(Alhadlaq and Mao, 2004)*: Bone marrow cells obtained from the long bones of 8 weeks old male albino rat by aspiration. Bones flushed with Dulbecco's Modified Eagle's medium (DMEM), (Sigma, USA, D5796) supplemented with 10% fetal bovine serum (FBS), (Sigma, USA, F6178). Bone marrow slowly layered over Ficoll- Hypaque (Sigma, USA, F8016) in a ratio of 2:1 in sterile conical tubes and was centrifuged (at1200 rpm for 30 minutes at room temperature). The opaque layer containing mononuclear cells was aspirated and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin (Sigma, USA, P4333). Cells were incubated at 37oC in 5% humidified CO2 for 14 days. Media were changed every 3~4 days. When

large colonies developed(80~90% confluence), cultures were washed twice with phosphate buffer saline (PBS) (P5493, Sigma, USA) and cells were trypsinized with 0.25% trypsin (Sigma, USA, T1426) in 1ml Ethylene Diamine Tetra Acetate (EDTA), (Sigma, USA,E6758) for 5 minutes at 37oC. After centrifugation (at 2400 rpm for 20 minutes at room temperature), cell pellets were resuspended with serum-supplemented medium and incubated in 25 cm2 culture flasks (Sigma, USA, C6356). The resulting cultures referred to as first-passage cultures. MSCs in culture were characterized by their plastic adhesiveness and fusiform shape ***(Rochefort , 2005)***.

4- Rats: Fifty adult male albino rats (total body weight, 150–200 g) were acclimated for one week prior to the experiment. Rats were housed in plastic cages, had free access to water and were given a semi-synthetic balanced diet with controlled temperature (21–23 C) and lighting (12h light/dark cycles). This study was approved by the Animal Experimentation Ethics Committee of the Egyptian National University.

II-Methods

1- Experimental design

Rats were divided into 5 groups with 10 rats per each group:

1. Group I (Control group): Rats were received 1 ml of normal saline (El-Nasr Company, Egypt) subcutaneously for 4 weeks.
2. Group II (Isoproterenol group): Isoproterenol was given by subcutaneous injection (85 mg/ kg.b.w.) once daily for two successive days.
3. Group III (Vit E– isoproterenol group): included 10 rats that were treated with ISO for 2 days as in group II, and after 1 week from the last dose of ISO, the animals received Vit E (100mg/kg b.w./ day) orally once daily for 1 week .
4. Group IV (Stem cell group): included 10 rats that were treated with ISO for 2 days as in group II, and after 1 week from the last dose of ISO, the animals received MSCs intravenously with 2x1o6 cells/rat once.
5. Group V (stem cell and vit. E group): included 10 rats that were treated with ISO and Vit. E as in group III, and after the last dose of Vit.E, the animals were injected with the MSCs intravenously.

2- Histological examination

The rats in each group were anesthetized with light ether inhalation and sacrificed one week after giving the last dose in each treatment protocol; thereafter, heart specimens were taken and fixed in10% formalin.

Then the formalin-fixed specimens were processed, embedded in paraffin wax and sliced at 4-6 µm thickness by a microtome. Then, sections were deparaffined, rehydrated and stained with Hematoxylin and eosin (Hx&E) ***(Bancroft and Gamble 2008)*** and Masson's trichrome (MT) ***(Leong 1996)***. Masson's trichrome stain was used to quantify the extent of fibrosis in the left ventricle (LV).

3- Immunohistochemical study

Immunohistochemistry to active caspase-3 was recently recommended for apoptosis detection Caspase 3 Immunohistochemical staining performed on 4-µm, formalin-fixed, paraffin-embedded sections using caspase 3 antibodies at 1:50 dilution (DAKO, Carpinteria, CA). Antigen retrieval was performed in all cases by steam heating the slides in a 1-mmol/L solution of EDTA (pH 8.0) for 30 minutes. After blocking of endogenous biotin, staining was performed using an automated immunostainer (DAKO) followed by detection by using a streptavidin-biotin detection system (DAKO). Analysis of tissue sections was performed by light microscopy.

CD105 immunostaining the marker for mouse mesenchymal stem cells. 0.1 ml prediluted primary antibody(CD105) rabbit polyclonal Ab (ab27422) and incubate at room temperature in moist chamber for 30~60 minutes. Tonsil used as positive control specimens. Cellular localization is the cell membrane. On the other hand, one of the heart sections was used as a negative control by passing the step of applying the primary antibody ***(Ramos-Vara 2005)***.

4- Morphometric study

The mean area percentage of collagen fibers deposition and Caspase immuno-expression was quantified in five images from five non-overlapping fields of each rat using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA).

5- Statistical analysis

The data collected from the experiment was recorded and analyzed using IBM SPSS Statistics software for Windows, Version 20 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) with Post Hoc LSD test was used to compare differences among the groups. In each test, the data was expressed as the mean (M) value, standard deviation (SD) and differences were considered to be significant at $P<0.01$.

RESULTS

In this study, the sections of rat heart of group I (control group) stained with H&E showed normal cardiac muscle with normal architecture and branching. They showed normal cardiac muscle cells with vesicular nuclei and acidophilic cytoplasm. Elongated , dark nuclei of fibroblasts were observed in the interstitial tissue between the muscle fibers (Figure 1). Sections of the heart of group II (Isoproterenol group) revealed marked separation of the cardiac muscle fibers, dilated and congested blood vessels with extravasation of blood cells between muscle fibers. Many pyknotic nuclei are noticed .There are also inflammatory cell infiltration, areas of degeneration of cardiac muscle fiber (Figure 2). Section of rat heart of group III (vit E and Isoproterenol), showed moderate separation of muscle fibers but still some blood vessels were dilated with extravasation of blood (RBCs) in between the cardiac muscle fibers. Some oedamtous separated heart fibers, some pyknotic nuclei as well as scattered areas of inflammatory cell infiltration were also seen (Figure 3). Group IV (Isoprotereno and stem cells) showed slight separation of muscle fibers, slight dilation of blood vessels with minimal extravasation of blood, and only few pyknotic nuclei (Figure 4).

In group V (Isoproterenol , stem cells and VitE), the cardiac sections restored its normal architecture but still few pyknotic nuclei were present with minimal extravasation of RBCs (Figure 5).

By using Masson's Trichrome stain (Figure 6), group V showed a minimal collagen deposition between the cardiac muscle fibers (Figure 10) in comparison to groups II, III ,IV (Figures 7-9). The degree of nuclear apoptosis was evaluated by immunohistochemical staining of Caspase-3. Positive caspase-3 immune reaction appeared as brown cytoplasmic staining (Figure 11). The more positive reaction means more apoptosis. Group V showed a very slight positive caspase-3 immune reaction in limited areas (Figure 15) when compared to groups II, III and IV (Figures 12-14).

CD 105: GroupI (control) showed a negative immunostaining in for CD 105 of cardiac muscle fibers (Figure16). Group IV (stem cell) showed a positive cytoplasmic reaction in regenerating cardiac muscle fiber (Figure 17). Group V (stem cell + vit E) showed more positive cytoplasmic reaction in regenerating cardiac muscle fiber (Figure 18).

Morphometric results

There was a significant decrease ($P<0.01$) in collagen fiber accumulation and in caspase-3 expression in groups III, IV and V compared with group II. There was also a significant decrease ($P<0.01$) in collagen fiber accumulation and in caspase-3 expression but insignificant decrease in caspase-3 expression in group V when compared to group III and IV (Tables 1 & 2, Histograms 1 & 2).

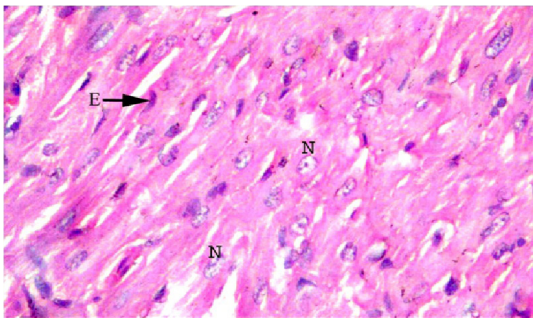


Fig. 1: A photomicrograph of cardiac muscle section of a rat from group I(Control group)(H&Es ×400)

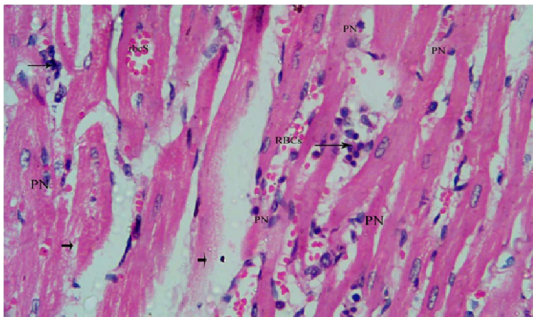


Fig. 2: A photomicrograph of cardiac muscle section of a rat from group II that received (ISO) (H&E ×400)

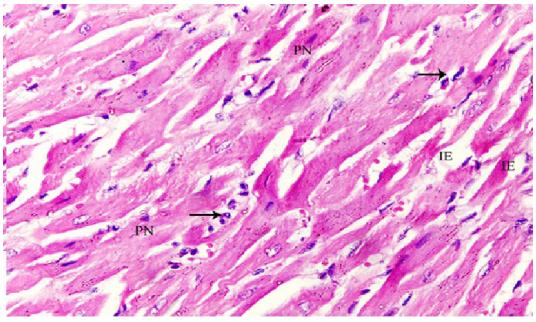


Fig. 3: A photomicrograph of cardiac muscle section of a rat from group III that receive vit E+ ISO (H&E ×400)

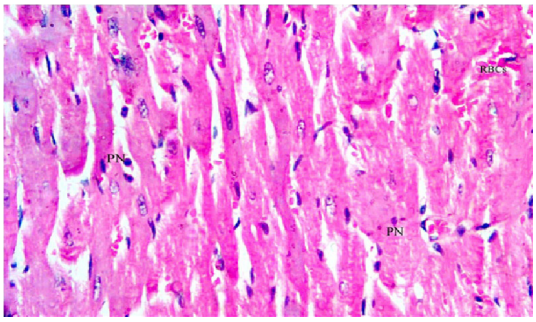


Fig. 4: A photomicrograph of rat cardiac muscle section from group VI receive (ISO + stem cell) (H&E x400)

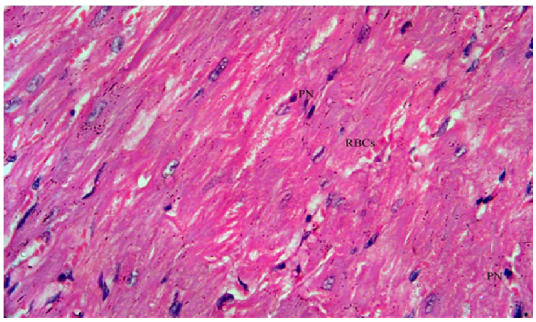


Fig. 5: A photomicrograph of cardiac muscle section of a rat from group V that receive (stem cell +Vit.E + ISO) (H&E x400)

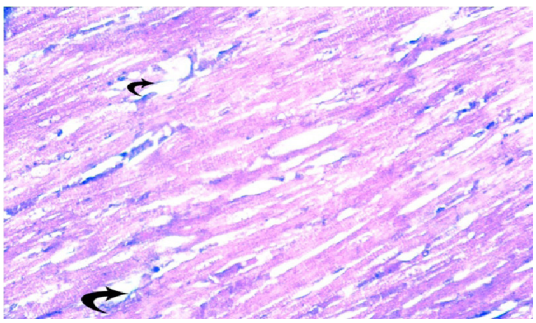


Fig. 6: A photomicrograph of cardiac muscle section of a rat from (control) group I (Masson,s trichome x 400)

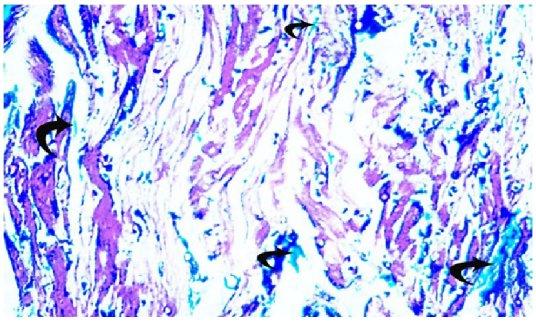


Fig. 7: A photomicrograph of cardiac muscle section of a rat from group II that receive ISO (Masson , s trichome x 400)

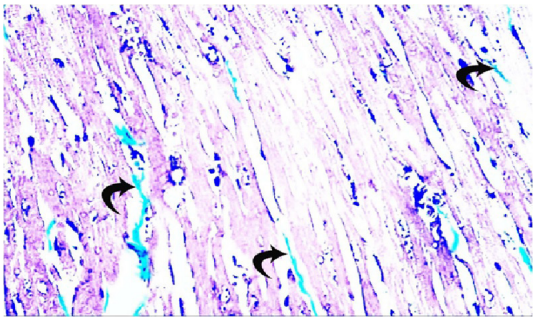


Fig. 8: A photomicrograph of cardiac muscle section of a rat from group III that receive ISO+ Vit. E (Masson,s trichome x 400)

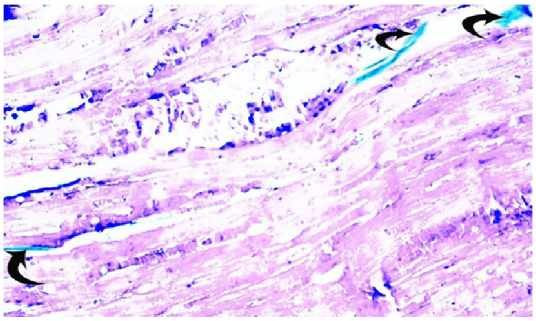


Fig. 9: A photomicrograph of cardiac muscle section of a rat from group IV that receive ISO+stem cell (Masson,s trichome x 400)

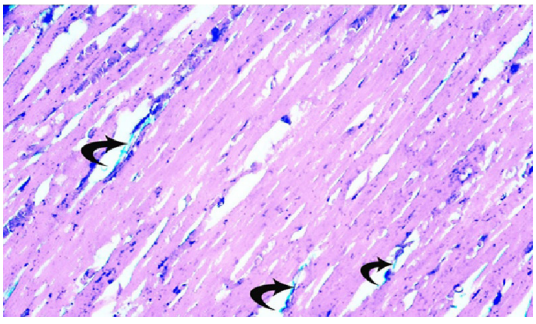


Fig. 10: A photomicrograph of cardiac muscle section of a rat from group V that receive ISO+stem cell+ vit E (Masson,s trichome x 400)

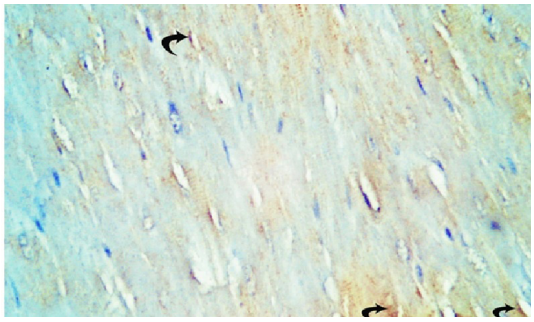


Fig. 11: A photomicrograph of cardiac muscle section of a rat from group I (control) (caspase- 3 x 400)

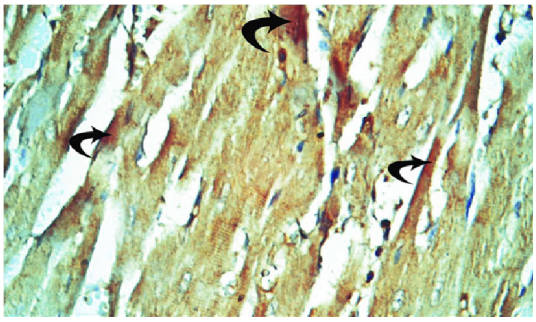


Fig. 12: A photomicrograph of cardiac muscle section of a rat from group II that received ISO (Caspas-3 x400)

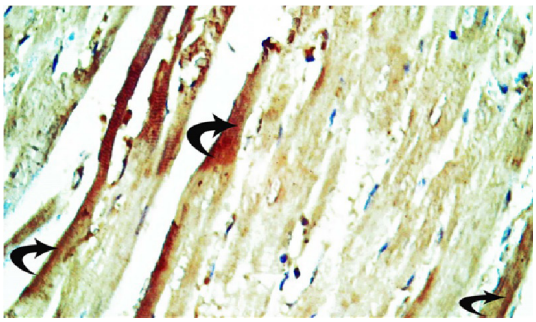


Fig. 13: A photomicrograph of cardiac muscle section of a rat from group III that received ISO + vit E (caspase- 3 x 400)

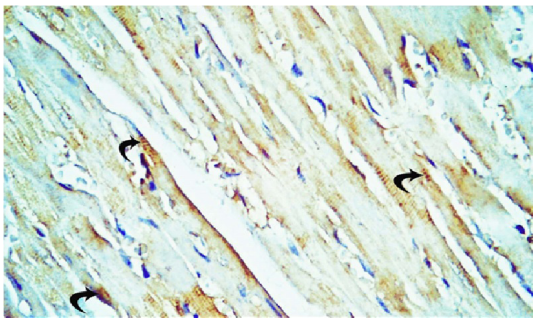


Fig. 14: A photomicrograph of cardiac muscle section of a rat from group III that received ISO + stem cell (caspase- 3 x 400)

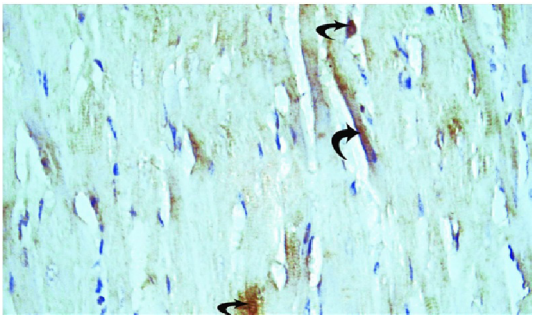


Fig. 15: A photomicrograph of cardiac muscle section of a rat from group V that received ISO + stem cell+ vit E (caspase- 3 x 400)

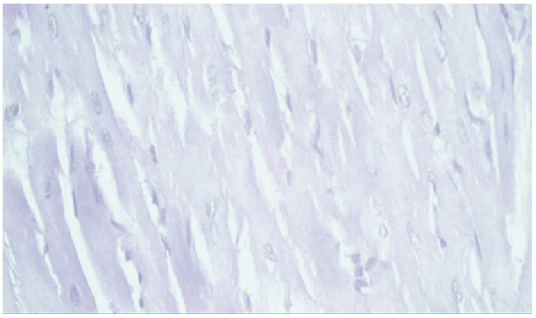


Fig. 16: A photomicrograph of cardiac muscle section of a rat from group I (control) (CD 105X 400)

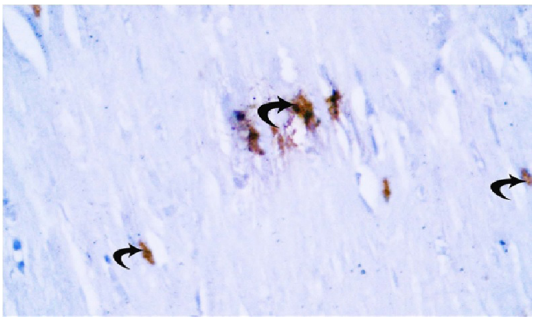


Fig. 17: A photomicrograph of cardiac muscle section of a rat from group IV that received ISO + stem cell (CD 105 X 400)

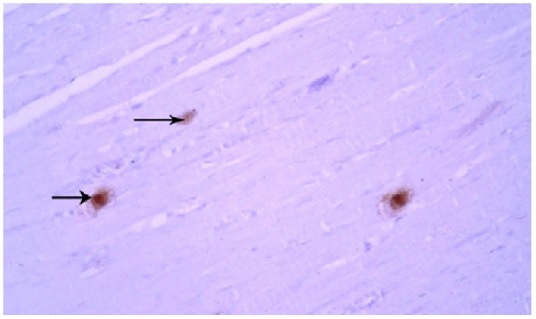
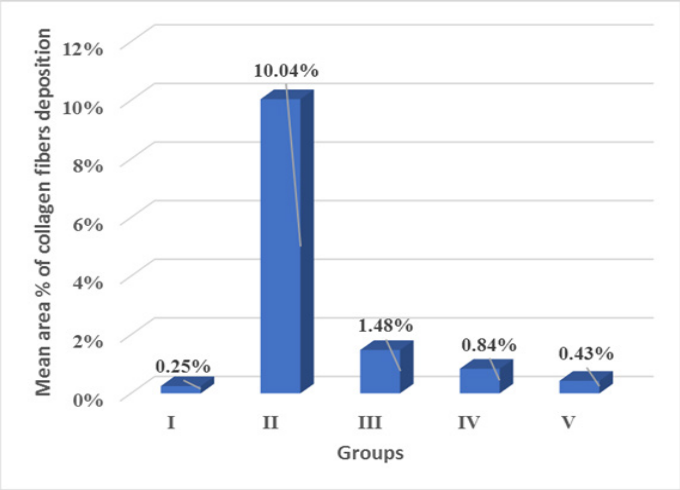


Fig. 18: A photomicrograph of cardiac muscle section of a rat from group V that received ISO+ stem cell+vit E) (CD 105 X 400)

Table 1: Showing the mean area %, SD of collagen fibers deposition in groups I, II, II, IV and V with comparison between all groups by Post Hoc LSD test

	Group I	Group II	Group III	Group IV	Group V
Mean area %	0.25%	10.04%	1.48%	0.84%	0.43%
SD	0.1436	0.5861	0.4017	0.0804	0.1763
Significance at P < 0.01	b,c,d	a,c,d,e	a,b,d,e	a,b,c,e	b,c,d

a=sig & group I b=sig & group II c=sig & group III d=sig & group IV e=sig & group V

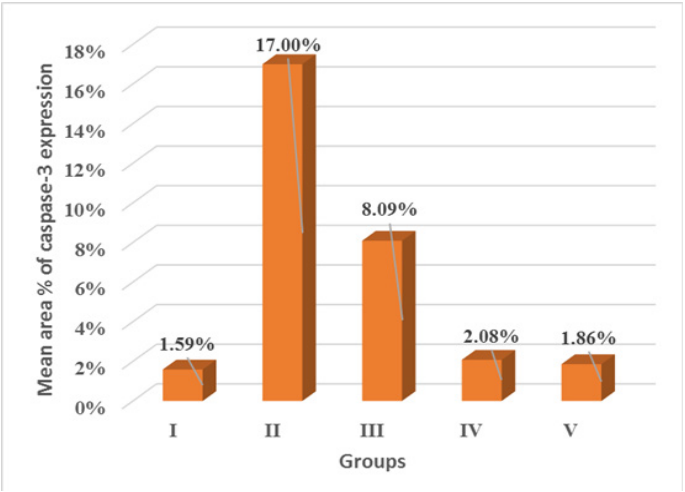


Histogram 1: Showing the mean area % of collagen fibers deposition in groups I, II, III, IV and V.

Table 2: Showing the mean area %, SD of caspase-3 expression in groups I, II, III, IV and V with comparison between all groups by Post Hoc LSD test

	Group I	Group II	Group III	Group IV	Group V
Mean area %	1.59%	17.00%	8.09%	2.08%	1.86%
SD	0.3965	0.5688	0.4017	0.0804	0.1936
Significance at P < 0.01	b,c,d	a,c,d,e	a,b,d,e	a,b,c	b,c

a=sig & group I b=sig & group II c=sig & group III d=sig & group IV e=sig & group V



Histogram 2: Showing the mean area % of caspase-3 expression in groups I, II, III, IV and V.

DISSCUSION

Isoproterenol injection was used in the present study because it is considered as an experimental model for studying myocardial infarction. This is in agreement with the previous study of ***(Murugesan et al, 2012)*** who reported that MI induced by ISO in experimental animal is characterized by many metabolic and morphological aberrations in the heart tissue similar to that observed in human MI. Mechanisms proposed to explain isoproterenol-induced cardiac damage include generation of highly cytotoxic free radicals that results from oxidative metabolism of catecholamine. These radicals are responsible for peroxidation of membrane phospholipids leading to permeability changes in the myocardial membrane.

Increased calcium overload, and mitochondrial injury or dysfunction are other possible mechanisms ***(Rathore et al.,2000, Punithavathi and Prince 2009 and Adámková et al., 2011)***.

In the present study, treatment with vitamin E showed a lesser degree of cardiac muscle fiber separation, necrosis, and inflammatory cell infiltration . vitamin E is a dietary compound, and its antioxidant properties are thought to be the common reason that it is pharmacologically useful against heart diseases. Vitamin E is a lipid soluble chain breaking antioxidant in human plasma and low density lipoprotein ***(Clark etal.,2008)***.

Vitamin E has been reported to produce a stabilizing effect on heart phospholipids by preventing changes in fatty acid composition . It could effectively trap the lipid peroxyl radical to inhibit the free radical initiated lipid peroxidation ***(Vivekananthan et al., 2003)***. It acts as the first line of defense against lipid peroxidation, protecting the cell membranes from free radical attack ***(Howard et al., 2011)***.

The elevation of reactive oxygen species and/ or decrease 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 was supported by previous reports mentioning the ability of Vit. E to maintain normal levels of antioxidant enzymes and to protect against oxidative tissue damage ***(Ithayarasi and Devi , 1997)***

The stem cell group (group IV) in the present study showed slightly disorganized cardiac muscle fibers, with cytoplasmic vacuolation and pyknosis in cardiomyocyte nuclei and a significant decrease (P<0.05) in collagen fiber accumulation, compared with group II. In agreement with these findings, ***(Ji et al., 2013)*** have shown that transplantation of autologous undifferentiated mesenchymal stem cells could be an effective method for myocardial regeneration after infarction, decreasing fibrosis, apoptosis, and left ventricular dilatation, while increasing myocardial thickness. The decreased amount of fibrosis after MSC injection was explained by ***(Wen et al., 2011)***, who mentioned that MSCs exert paracrine antifibrotic effects to attenuate ventricular remodeling through regulation of cardiac fibroblast proliferation.

Intravenous infusion of allogenic MSCs in humans with acute MI revealed fewer ventricular arrhythmias than in those with placebo infusion ***(Hare et al., 2009)*** .These studies revealed that intravenous allogenic MSCs are safe in patients with acute MI. Likewise, MSC therapy in other clinical trials was not associated with any adverse effects ***(Chen etal.,2004 and Williams etal.,2011)***

In the present study when Vit. E combined with stem cells were given in MI (group V), the myocardium showed nearly a back to normal morphological architecture that may confirm the cardioprotective effects of Vit. E and stem cells . These findings were in agreement with those of ***(Urish et al., 2009)*** who mentioned that antioxidant levels could significantly affect cell behavior, stem cell characteristics, and survival. Cell viability possesses a major obstacle for any cell based therapeutic strategy in the infarcted heart ***(Song et al.,2010)***

Reactive oxygen species (ROS) is known to be a key mediator in cardiac dysfunction. ROS is known to hinder cell adhesion and stimulate cell detachment and death ***(Zhu et al., 2009)***. The grafted cell may encounter ischemic conditions, lack of nutrients and oxygen and consequently affecting cell viability (Mylotte et al., 200***8)***. On the other hand, myocardial injury has been shown to generate a strong inflammatory response followed by production of oxygen-derived free radicals and inflammatory cytokines that trigger cell death and initiate apoptosis (Frangogiannis 2006). Thus, increasing the cellular antioxidant levels by giving Vit.E before transplantation could further increase stem cell survival and thereby improve functional repair.

CONCLUSION

we can conclude that if either Vit.E preparations or stem cells are given alone after

myocardial infarction, some improvement of myocardial fibers occurs but when they are given together (VitE. and stem cells), better results are obtained .

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

Adámková V, Kacer P, Mraz J, Suchanek P, Pickova J, Králova Lesná I, Skibova J, Kozak P, Maratka V (2011) : The consumption of the carp meat and plasma lipids in secondary prevention in the heart ischemic disease patients. Neuro. Endocrinol. Lett. 32 Suppl. 2:17-20.

Alhadlaq A and Mao JJ. (2004): Mesenchymal stem cells: isolation and therapeutics. Stem Cells Dev 13:436-448

Assmus B and Zeiher AM. (2013): Early cardiac retention of administered stem cells determines clinical efficacy of cell therapy in patients with dilated cardiomyopathy. Circ Res 112:6-8.

Aman Upaganlawar and Ramachandran Balaraman(2009): Combined Effect of Green Tea Extract and Vitamin E on Serum and Heart Tissue Lipids, Lipid Metabolizing Enzymes and Histopathological Alteration in Isoproterenol-InducedMyocardial Infarction in Rats. Sci Pharm. 77: 791–803

Bancroft J. D. and Gamble M. (2008): Theory and practice of histological techniques. 6th ed. Churchill Livingstone. London, New York & Sydney. P. 121-132.

Bartunek J, Behfar A, Dolatabadi D (2013): Cardiopoietic stem cell therapy in heart failure: the C-CURE (Cardiopoietic stem Cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics. J Am Coll Cardiol;61:2329-38.

Burn BR, Varner KJ. (2015): Environmentally persistent free radicals compromise left ventricular function during ischemia/reperfusion injury. Am. J. Physiol. Heart Circ. Physiol. 308(9):H998-H1006.

Clark MW, Burnett JR, Croft KD. (2008): Vitamin E in human health and disease. Crit Rev Clin Lab Sci. 45: 417–450.

Frangogiannis N. G., (2006): "Targeting the inflammatory response in healingmyocardial infarcts," Current Medicinal Chemistry, vol. 13, no. 16, pp. 1877–1893.

Go AS, Mozaffarian D, Roger VL (2013): Heart disease and stroke statistics--2013 update: a report from the American Heart Association. Circulation;127: e6-e245.

Hare J. M., Traverse J. H.and Henry T. D. (2009) : "A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction," Journal of the American College of Cardiology, vol. 54, no. 24, pp. 2277– 2286.

Howard AC, Anna K, McNeil AK, McNeil PL. (2011): Promotion of plasma membrane repair by vitamin E. Nat Commun.;20:597.

Ithayarasi AP1, Devi CS. (1997): Effect of alpha-tocopherol on lipid peroxidation in isoproterenol induced myocardial infarction in rats. Indian J Physiol Pharmacol. Oct 41(4):369-76.

Jeevanantham V, Butler M, Saad A (2012): Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. Circulation 126:551-68.

Ji L, Long X, Tian H, Liu Y. (2013): Effect of transplantation of bone marrow stem cells on myocardial infarction size in a rabbit model. World J Emerg Med 4:304–310.

Kannan MM, Quine SD (2013): Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidaemia during myocardial infarction in rats. Metabolism 62:52–61.

Leong, A. S. (1996): Principles and practice of medical laboratory science. Volume 1: Basic Histotechnology. 1st ed., Philadelphia, Saunders Company. P. 171.

Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, (2012): Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet 379(9819):895–904.

Mehdizadeh R, Parizadeh MR, Khooei AR, Mehri S, Hosseinzadeh H.(2013): Cardioprotective effect of saffron extract and safranal in isoproterenol induced myocardial infarction in wistar rats. Iran J Basic Med Sci 16:56–63.

Mukesh Nandave, Ipseeta Mohanty, T. C. Nag, Shreesh Kumar Ojha, Rajan Mittal, Santosh Kumari2, Dharamvir Singh Arya (2007): cardioprotective response to chronic administration of vitamin e in isoproterenol induced myocardial necrosis: hemodynamic, biochemical and ultrastructural studies. indian journal of clinical biochemistry, 22 (1) 22-28

Munteanu a, zingg jm, azzi a. (2004): anti-atherosclerotic effects of vitamin e- myth or reality. j cell mol med; 8: 59-76.

Murugesan M, Ragunath M, Prabu T, Nadanasabapathi S, Sakthivel M, Manju V. (2012): Protective role of black cumin (Nigella sativa) on isoproterenol induced myocardial infarction in rats. Int J Pharmacol Clin Sci 1: 45–53.

Mylotte L. A., Duffy A. M. and Murphy M. (2008): "Metabolic flexibility permits mesenchymal stem cell survival in an ischemic environment," Stem Cells, vol. 26, no. 5, pp. 1325–1336.

Punithavathi VR, Prince PS. (2009): Combined effects of quercetin and α-tocopherol on lipids and glycoprotein components in isoproterenol induced myocardial infarcted Wistar rats. Chem Biol Interact 181:322-7.

Ramos-Vara, J. A. (2005). "Technical Aspects of Immunohistochemistry". Veterinary Pathology. 42 (4): 405–426..

Rathore N, Kale M, John S, Bhatnagar D. (2000): Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat erythrocytes. Indian J Physiol Pharmacol 44:161–6.

Rochefort GY, Vaudin P, Bonnet N, Pages JC, Domenech J, Charbord P, Eder V. (2005): Influence of hypoxia on the domiciliation of mesenchymal stem cells after infusion into rats: possibilities of targeting pulmonary artery remodeling via cells therapies? Respir Res 6:125

saldeen t, li d. mehta, jl. (1999): different effects of alpha- and gamma-tocopherol on ldl oxidation, superoxide activity, platelet aggregation and arterial thrombogenesis. j am coll cardiol; 34: 1208-15

Song H . , Song B.W. ,Cha M. J., Choi I.G. and Hwang K.C.(2010): Modification of mesenchymal cells for cardiac regeneration .Expert opinion on biological Therapy,vol.10,no 3,pp.309-319

Steinhauser ML, Lee RT. (2011) Regeneration of the heart. EMBO Mol Med 3 :701-12.

Sürder D, Manka R, Lo Cicero V, Moccetti T, Rufibach K, Soncin S (2013): Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function. Circulation 127(19):1968–79.

Urish KL, Vella JB, Okada M, Deasy BM, Tobita K, Keller BB,(2009): Antioxidant levels represent a major determinant in the regenerative capacity of muscle stem cells. Mol Biol Cell; 20:509–520.

Vivekananthan DP1, Penn MS, Sapp SK, Hsu A, Topol EJ (2003): Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet. Jun 14;361(9374):2017-23.

Wen Z, Zheng S, Zhou C, Wang J, Wang T. (2011): Repair mechanisms of bone marrow mesenchymal stem cells in myocardial infarction. J Cell Mol Med 15:1032–1043.

Williams A. R., Trachtenberg B.and Velazquez D. L. (2011) : "Intramyocardial stem cell injection in patients with ischemic cardiomyopathy: functional recovery and reverse remodeling," Circulation Research, vol. 108, no. 7, pp. 792–796.

Zhu W. G, Li S., Lin L. Q., Yan H., Fu T., and Zhu J. H.(2009): "Vascular oxidative stress increases dendritic cell adhesion and transmigration induced by homocysteine," Cellular Immunology, vol. 254, no. 2, pp. 110–116.

التأثيرات التعزيزيه المحتملة لفيتامين ه على علاج بالخلايا الجذعية الوسيطة لاحتشاء القلب الناتج عن الايزوبروتيرينول فى ذكور الجرذان البيضاء

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ملخص البحث

المقدمة : يتعرض الناجين من احتشاء عضلة القلب الي تندب تليها اعادة هيكلة البطين علي الرغم من الرعايه الطبيه المثلى. وقد اجتذب العلاج القائم على الخلايا الجزعيه مزيدا من الاهتمام من حيث مساهمته المحتمله فى تجديد القلب والاوعيه الدمويه. ومع ذلك ، فان امكانية العلاج يعوقها معدل بقاء هذه الخلايا على قيد الحياة بعد زرعها فى عضلة القلب التالفة.

الهدف من الدراسة الحالية: معرفة ما اذا كان فيتامين ه يمكن ان يعزز فاعلية العلاج بالخلايا الجزعية الوسيطه للقلب بعد تعرضه للايسوبروتيرينول الذى يسبباحتشاء عضلة القلب فى الفئران

المواد والطرق : تم تقسيم خمسين جرذا من الذكور البيضاء البالغة إلى 5 مجموعات متساوية (10 لكل مجموعة) المجموعة الأولى (المجموعة الضابطة): تم إعطاء كل جرذ 1 ملى من الماء المقطر لمدة اربع اسابيع , المجموعة الثانية (مجموعة الايزوبروتيرينول): تم إعطاء كل جرذ85 ملجرام/كجرام من عقار الايزوبروتيرينول مره واحده يوميا لمدة يومين متتاليين ، المجموعة الثالثة (مجموعةايزوبروترينول وفيتامين ه) : تم إعطاء كل جرذ85 ملجرام/كجرام من عقار الايزوبروتيرينول مره واحده يوميا لمدة يومين متتاليين وبعد مرور اسبوع تم اعطاؤهم فيتامين ه بجرعة 100ملجرام/كجرام من وزن الجسم عن طريق الفم لمدة اسبوع المجموعة الرابعة علجت الجرذان بالايزوبروترينول كما حدث بالمجموعة الثانيه وبعد مرور اسبوع اعطيت جرعة واحدة من الخلايا الجزعية تعادل2×610 لكل جرذ عن طريق الحقن بالوريد وفى المجموعة الخامسة اعطيت الجرذان ايزوبروتيبرينول وفيتامين ه كما فى المجموعة الثالثة وبعد اخر جرعة اعطيت الخلايا الجزعية كما فى المجموعة الرابعة. وقد اخذت العينات من القلب واعدت للفحص باستخدام التقنيات الهستولوجيه والهستوكيميائيه المناعيه.

النتائج: كشفت النتائج أن مجموعة الايزوبروتيرينول قد أظهرت عدم انتظام وانفصال واسع بين الألياف العضلية مع تجويفات سيتوبلازميه وتغلظ في انوية العديد من خلايا القلب و تسرب لخلايا الدم الحمراء. واظهرت مجموعة فيتامين ه تجويفات سيتوبلازميه وتغلظ في انوية بعض من خلايا القلب . اما مجموعة الخلايا الجذعية فقد أظهرت عدم انتظام بسيط بين الألياف العضلية للقلب مع تجويفات سيتوبلازميه وتغلظ في انوية القليل من خلايا القلب . هذا وقد اظهرت مجموعةالخلايا الجذعية وفيتامين ه شكل طبيعي لترتيب الياف القلب والتركيب الدقيق لعضلة القلب ما عدا القليل من الانفصال بين بعض حزم الالياف الصغيرة .كما اظهرت هذه المجموعة انخفاض ذو تاثيرفى تراكم الياف الكولاجين وفي تفاعل بروتين كسباس3مقارنة بمجموعة فيتامين ه ومجموعة الخلايا الجذعية وعن طريق الفحص الهستوكيميائى المناعى تبين وجود الخلايا الجزعيه مع وجود رد فعل سيتوبلازميا أكثر إيجابية في تجديد عضلة القلب فى مجموعةالخلايا الجذعية وفيتامين ه .

الخلاصة: ايمكن ان نستنتج ان اعطاء فيتامين ه او الخلايا الجزعية كل على حدى فى حالات احتشاء القلب يؤدى الى تحسن بينما اعطاء فيتامين ه مع الخلايا الجزعيه معا يعطى نتائج افضل كثيرا