**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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**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 26 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) 30 and Masson’s Trichrome stains .Also Immunohistochemical study was done to 31 detect caspas-3 and CD105. Morphometric study: The mean area percentage ofcollagen 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 2 separation of cardiac muscle fibers and extravasation of blood. In ISO &stem cell 3 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( Vit E. And stem cells), better results are obtained

 **Keyword:** Acute myocardial infarction- Isoproterenol - Stem cells-Vit.E- Caspas 3

**INTRODUCTION:-** Cardiovascular disease (CVD) is one of the main causes of death( From 1999 to 2009) . The 23 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 26 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 ofcurrent pharmacological, interventional or operative therapies is their disability to compensatethe irreversible loss of functional cardiomyocytes ( Steinhauser andLee.,2011) Hence, the future challenge of cardiovascular therapies will be the functional regeneration of myocardial 31 contractility by novel concepts, like cell based therapy, tissue engineering or reprogramming of scar fibroblasts ( Assmus and Zeiher .,2013) 33 34 During the past decade, many clinical trials showed positive results of cell therapy (Makkar 35 et al.,2012) ,while other clinical studies showed no beneficial effect of cell therapy over placebo 36 (Sürder et al., 2013). Stem-cell-based therapy has been given increased attention in terms of its potential 38 contribution to cardiovascular regeneration. Previously published data showed that mesenchymal stem cells (MSCs) had been widely applied in regenerative medicine and 2 exhibited beneficial effects on postinfarct hearts (Bartunek, et al.,2013). However, the 3 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). 6 Isoproterenol, a beta-adrenoceptor agonist, has been reported to produce MI in large 7 doses.Upon auto-oxidation, isoproterenol generates highly cytotoxic free radicals known to 8 stimulate the peroxidation of membrane phospholipids causing severe damage to the 9 myocardial membrane. Hence, it is widely used as a model to produce myocardial infarction in 10 rats (Kannan andQuine.,2013) . 11 Vitamin E (vit E) is the most widely used vitamin in food Supplements . Owing to its wide array 12 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 14 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 21 stem cell treatment of isoproterenol-induced myocardial Infarction in rats

**Materials and methods: 23 I-Materials** : - 24 1-Isoproterenol (ISO) hydrochloride was purchased in the form of a white powder from 25 Sigma Chemical Company. It was administered subcutaneously daily at a dose of 85 mg/kg b.w. 26 dissolved in 5 ml of normal saline (0.9% NaCl) for 2 days (Mehdizadeh etal.,2013) . 27 2- Vitamin E is available commercially as E–Viton capsules produced by Kahira Pharm. and 28 Chem.Ind. Company. Each capsule contains 100 mg α-tocopherol acetate. The recommended 29 dose of vitamin E in rats is 100mg/kg/day. The content of one capsule was dissolved in 30ml 30 corn oil ( Aman and Ramachandran, 2009) . 31 3-Isolation, culture and labeling of MSCs from rat bone marrow (Alhadlaq and 32 Mao, 2004) : Bone marrow cells obtained from the long bones of 8 weeks old male albino rat by 33 aspiration. Bones flushed with Dulbecco's Modified Eagle's medium (DMEM) ,(Sigma, USA, 34 D5796) supplemented with 10% fetal bovine serum (FBS), (Sigma, USA, F6178). Bone marrow 35 slowly layered over Ficoll- Hypaque (Sigma, USA, F8016) in a ratio of 2:1 in sterile conical tubes 36 and was centrifuged (at1200 rpm for 30 minutes at room temperature). The opaque layer 37 containing mononuclear cells was aspirated and resuspended in complete culture medium 4 1 supplemented with 1% penicillin-streptomycin (Sigma, USA, P4333). Cells were incubated at 2 37oC in 5% humidified CO2 for 14 days. Media were changed every 3∼4 days. When large 3 colonies developed(80∼90% confluence), cultures were washed twice with phosphate buffer 4 saline (PBS) (P5493, Sigma, USA) and cells were trypsinized with 0.25% trypsin (Sigma, USA, 5 T1426) in 1ml Ethylene Diamine Tetra Acetate (EDTA) , (Sigma, USA,E6758) for 5 minutes at 6 37oC. After centrifugation (at 2400 rpm for 20 minutes at room temperature), cell pellets were 7 resuspended with serum-supplemented medium and incubated in 25 cm2 culture flasks (Sigma, 8 USA, C6356). The resulting cultures referred to as first-passage cultures. MSCs in culture were 9 characterized by their plastic adhesiveness and fusiform shape (Rochefort , 2005). 10 4-Rats : 11 Fifty adult male albino rats (total body weight, 150–200 g) were acclimated for one week prior 12 to the experiment. Rats were housed in plastic cages, had free access to water and were given a 13 semi-synthetic balanced diet with controlled temperature (21–23 C) and lighting (12 h 14 light/dark cycles). This study was approved by the Animal Experimentation Ethics Committee of 15 the Egyptian National University. 16 II-Methods :- 17 1-Experimental design:- 18 Rats were divided into 5 groups with 10 rats per each group: 19 (1)Group I (Control group): Rats were received 1 ml of normal saline (El-Nasr Company, Egypt) 20 subcutaneously for 4 weeks. 21 (2)Group II (Isoproterenol group): Isoproterenol was given by subcutaneous injection( 85 mg/ 22 kg.b.w. ) once daily for two successive days. 23 (3)Group III (Vit E– isoproterenol group): included 10 rats that were treated with ISO for 2 days 24 as in group II, and after 1 week from the last dose of ISO, the animals received Vit E(100mg/kg 25 b.w./day) orally once daily for 1 week . 26 (4) Group IV (Stem cell group): included 10 rats that were treated with ISO for 2 days as in 27 group II, and after 1 week from the last dose of ISO, the animals received MSCs intravenously 28 with 2x1o6 cells/rat once. 29 (5) Group V (stem cell and vit. E group) : included 10 rats that were treated with ISO and Vit. E 30 as in group III, and after the last dose of Vit.E, the animals were injected with the MSCs 31 intravenously. 32 2- Histological examination : The rats in each group were anesthetized with light ether 33 inhalation and sacrificed one week after giving the last dose in each treatment protocol; 34 thereafter, heart specimens were taken and fixed in10% formalin. 35 Then the formalin-fixed specimens were processed, embedded in paraffin wax and sliced at 4-6 36 µm thickness by a microtome. Then, sections were deparaffined, rehydrated and stained with 37 Hematoxylin and eosin (Hx &E) [Bancroft and Gamble2008] and Masson's trichrome (MT) 5 1 [Leong 1996] . Masson’s trichrome stain was used to quantify the extent of fibrosis in the left 2 ventricle (LV). 3 3-Immunohistochemical study : 4 Immunohistochemistry to active caspase-3 was recently recommended for apoptosis detection 5 Caspase 3 Immunohistochemical staining performed on 4-μm, formalin-fixed, paraffin6 embedded sections using caspase 3 antibodies at 1:50 dilution (DAKO, Carpinteria, CA). Antigen 7 retrieval was performed in all cases by steam heating the slides in a 1-mmol/L solution of EDTA 8 (pH 8.0) for 30 minutes. After blocking of endogenous biotin, staining was performed using an 9 automated immunostainer (DAKO) followed by detection by using a streptavidin-biotin 10 detection system (DAKO). Analysis of tissue sections was performed by light microscopy. 11 CD105 immunostaining the marker for mouse mesenchymal stem cells. 0.1 ml prediluted 12 primary antibody(CD105) rabbit polyclonal Ab (ab27422) and incubate at room temperature in 13 moist chamber for 30∼60 minutes. Tonsil used as positive control specimens. Cellular 14 localization is the cell membrane. On the other hand, one of the heart sections was used as a 15 negative control by passing the step of applying the primary antibody ( Ramos-Vara 2005). 16 4-Morphometric study: - 17 The mean area percentage of collagen fibers deposition and Caspase immuno-expression was 18 quantified in five images from five non-overlapping fields of each rat using Image-Pro Plus 19 program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA). 20 5-Statistical analysis: - 21 The data collected from the experiment was recorded and analyzed using IBM SPSS Statistics 22 software for Windows, Version 20 (IBM Corp., Armonk, NY, USA). One-way analysis of variance 23 (ANOVA) with Post Hoc LSD test was used to compare differences among the groups. In each 24 test, the data was expressed as the mean (M) value, standard deviation (SD) and differences 25 were considered to be significant at 𝑃 < 0.01..