INTRODUCTION

Cardiovascular disease is one of the major problems worldwide and was considered as one of the leading causes of deaths in *2008* (17 million deaths). Over 80% of deaths occurred in the low and middle income countries ***(WHO, 2011)***. Myocardial infarction (MI) is considered to be one of the main causes of death from cardiovascular disease ***(Gupta et al., 2013).***

In developed countries, acute myocardial infarction accounts for 10-25% of all deaths ***(Mohan, 2010).*** It is defined as an acute necrosis of the myocardium that occurs as a result of an imbalance between coronary blood supply and myocardial demand ***(Gupta et al., 2013).***

The ability of the heart for regeneration has for long been settled to be a terminally differentiated organ ***(Soonpaa and Field, 1998).*** On the other hand, ***Kudo et al. (2003)*** demonstrated that cultured bone marrow stromal cells reduced the infarction and fibrosis in ischemic heart**.** Moreover, ***Halkos et al., (2008)*** suggested that intravenous infusion of mesenchymal stem cells was effective in treatment of ischemia reperfusion induced myocardial injury.

Some studies have been conducted to investigate the possible role of stem cells in the regeneration of the injured myocardium ***(Kollar et al., 2009).***

In the recent few years a new type of interstitial cells has been discovered which was termed telocyte. Telocytes have been detected in many organs including the skeletal muscle ***(Popescu et al., 2011)***, lung ***(Zheng et al., 2011)***, heart ***(Gherghiceanu and Popescu, 2012)***, kidney ***(Guisheng et al., 2012)*** and skin ***(Ceafalan et al., 2012)***.

Zheng et al. (2013) reported that the exact function of telocytes is still not clear and unknown. They suggested that the gene signature of telocytes proposes a specific role in tissue development and morphogenesis and angiogenesis.

Aim of the Work

The aim of this study is to assess the efficacy of bone marrow mesenchymal stem cells injection, in the repair of an experimentally induced myocardial infarction. In addition, a special focus will be directed towards the role of telocytes in myocardial regeneration.

HISTOLOGY OF THE HEART

The heart is a four-chambered organ composed of two atria and two ventricles. These chambers possess common characteristics in that they are composed of three main layers: epicardium, myocardium and endocardium.

The **epicardium** is the outermost layer. It is covered by a simple squamous mesothelium. Deep to it, is a fibroelastic connective tissue. The deepest aspect of the epicardium is composed of adipose tissue that has nerves and the coronary vessels ***(Ross and Pawlina, 2016).***

The **myocardium**, compose most of the heart thickness. It consists of branched bundles of cardiac muscle that are attached to the thick collagenous connective tissue skeleton of the heart.The cardiac muscle cells are cylindrical in shape and striated. Each cell usually contains one (or two) centrally placed nucleus. Surrounding the muscle cells is a delicate sheath of endomysium with a rich capillary network. The myofibrils of cardiac muscle separate to pass around the nucleus. The juxtanuclear region contains the cell organelles as it is rich in mitochondria which occupy 40% or more of the cytoplasmic volume and also contains the Golgi apparatus, lipofuscin pigment granules, and glycogen. The cardiac muscles are characterized by large mitochondria that are densely packed between the myofibrils. These large mitochondria contain numerous, closely packed cristae. The smooth endoplasmic reticulum of cardiac muscle is not as well organized ***(Ross and Pawlina, 2016)***.

The cardiac muscle cells are connected by specialized junctions known as intercalated discs which appear densely stained in light microscopic (LM) examination. Using TEM, each intercalated disc has a transverse portion (numerous desmosomes and fasciae adherents) and lateral portions that are rich in gap junctions which are oriented in a step like fashion ***(Gartner and Hiatt, 2014).***

The **endocardium** forms the lining of the atria and ventricles and is composed of a simple squamous endothelium as well as a subendothelial fibroelastic connective tissue. The endocardium participates in the formation of the heart valves. The **subendocardial layer** is a deep layer of connective tissue that merges with the myocardium. Branches of the conducting system, consisting of modified cardiac muscle fibers, are also located in the subendocardial layer ***(Mescher, 2013).***

The heart valves are composed of connective tissue with overlying endocardium. Each valve is composed of three layers; fibrosa, spongiosa and ventricularis ***(Ross and Pawlina, 2016).***

MYOCARDIAL INFARCTION

Cardiovascular disease (CVD) is a major worldwide health problem and was considered as one of the leading causes of deaths in *2008*. Over 80% of deaths occurred in the low and middle income countries ***(WHO, 2011)***.

Predisposing factors for CVD which increase its prevalence include hypertension, dyslipidemia, diabetes, obesity, physical inactivity and tobacco smoking ***(Upaganlawar et al., 2011).***

Cardiovascular diseases include increased blood pressure, coronary heart disease, congestive heart failure and stroke ***(Reeve et al., 2005).***

The prevalence of the classical coronary artery disease risk factors has reached alarming rates in some developing countries. Acute myocardial infarction (AMI) in particular remains one of the leading causes of death in the developing world ***(Mouhamad et al., 2006)*** as well as in the developed world as it accounts for 10-25% of all deaths ***(Mohan, 2010).*** Myocardial infarction (MI), commonly known as heart attack is considered to be one of the main causes of death from CVD ***(Gupta et al., 2013).***

Following AMI 80-90% of cases develop one or more major complications which may be fatal. Arrhythmias, congestive heart failure, cardiogenic shock, thromboembolism, rupture of the heart and cardiac aneurysms ***(Mohan, 2010).***

Myocardial infarction is defined as myocardial necrosis due to prolonged ischaemia. The ischemia can be due to a decrease in the blood supply to the heart as in coronary artery occlusion. It is manifested clinically by varying degrees of chest pain, weakness, sweating, vomiting, arrhythmias and sometimes loss of consciousness up to sudden death ***(Kumar and Jugdutt, 2003).***

After the onset of myocardial ischaemia, cell death is not immediate but takes a certain period to develop (20 min or less in some animal models). It takes several hours before myocardial necrosis could be identified by macroscopic or microscopic post-mortem examination**.** Myocardial infarction can be classified pathologically after death as acute, healing, or healed. *Acute myocardial infarction,* (starting from 6 h–7days) is characterized by the presence of polymorph nuclear leukocytes. If the time interval between the onset of the infarction and death is too brief, so minimal or no polymorph nuclear leukocytes may be seen. *Healing infarction* is characterized by the presence of mononuclear cells and fibroblasts, and the absence of polymorph nuclear leukocytes (from 7–28 days). *Healed infarction* is manifested as scar tissue without cellular infiltration (29 days and beyond) ***(Thygesen et al., 2012).***

Mohan, in 2010 stated that, in the first 6 hours of myocardial infarction no histological changes were detected except some waviness in myocardial fibers. After 6 hours, edema, fluid in

between the fibers and the fibers showed degeneration. Starting from 12 hours, coagulative necrosis was detected in the form of loss of striations, intense eosinophilia of the cytoplasm, hyaline appearance and nuclear (pyknosis, karyolysis and karyorrhexis). Cellular infiltration mainly neutrophils, hemorrhage and edema in the interstitium developed**.**

Biomarker Evaluation of acute myocardial infarction:

Myocardial cell death can be recognized by the release of different proteins into the circulation from the damaged myocytes, including: myoglobin, cardiac troponin T and I, ceriatine kinase (CK), lactate dehydrogenase (LDH) ***(Mendis et al., 2011).***

Acute myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers such as cardiac troponin or cardiac ceriatine kinase-MB (CK-MB) are increased. Cardiac troponin (I or T), actually has high myocardial tissue specificity as well as high clinical sensitivity ***(Mendis et al., 2011).*** Troponin values may remain elevated for 7–14 days following the onset of infarction.For more confirmation, CK-MB isoenzyme is used as it is more specific than CK as a whole ***(Thygesen et al., 2012).***

Isoproterenol induced myocardial infarction:

Isoproterenol (ISO) is a synthetic catecholamine and adrenergic agonist stimulating beta 1 and beta 2 adrenergic receptors, but has no effect on alpha receptor capabilities ***(Karthick and Stanely, 2006).***

Isoproterenol had been used as a drug to induce myocardial infarction in rats ***(Acikel et al., 2005; Rajadurai and Stanely, 2006; Zhang et al., 2008).***

Different routes of ISO had been used either intraperitoneal ***(Choudhary et al., 2006)***, or subcutaneously ***(Gupta et al., 2015)*** for induction of myocardial infarction**.**

Induction of myocardial infarction in male rats was done, by injection of ISO (200 mg/ kg) subcutaneously at an interval of 24 h for two days. After 48 h specimens were examined for cardiac injury which appeared as molted staining with fragmentation of muscle fibers ***(Thippeswamy et al., 2009)***.

Isoproterenol -induced acute myocardial injury appeared as early as 1–3 hours in rats after single injection of ISO intraperitoneally ***(Dudnakova et al., 2003 and Goldspink et al., 2004).*** High levels of ISO were proved to be cardiotoxic and influence cardiac cells in a way that resembled changes observed in MI in humans. Both biochemical and histopathologic changes were observed with an increase in cardiac marker enzymes and DNA damage in rats as indicated by increased level of 7,8-Dihydro-8-oxo-guanine (8-OHGua)in

blood, which was an indicator of DNA damage ***(Nair and Shyamala, 2006 and Keles et al., 2009).***

Histologically ISO induced myocardial membrane damage, extensive myonecrosis, vascular changes, fibroblastic proliferation as well as oedema ***(Zhuo et al., 2013).*** Also ISO caused cardiac hypertrophy in rats ***(Choudhary et al., 2006).***

Recently, ISO-induced heart damages were related to several signaling pathways, such as oxidative stress, calcium dysregulation, and activation of apoptosis gene ***(Liu et al., 2015).***

THE STEM CELLS

Stem cells are unspecialized cells with the ability to self-renew, differentiate into one or more specialized cell types playing a critical role in homeostasis and tissue repair. Based on their origin, stem cells were categorized either as embryonic stem cells or adult stem cells ***(Nadig, 2009).***

Adult stem cells are found in most adult tissues. They are multipotent capable of differentiating into more than one cell type but not all cell types. Adult stem cells can be further classified as hemopoetic stem cells and mesenchymal stem cells (MSCs). Hemopoetic stem cells are obtained either from cord blood or peripheral blood. Mesenchymal stem cells are those that originate from the mesoderm layer of the fetus and in the adult, reside in a variety of tissues such as the bone marrow stem cells, limbal stem cells, hepatic stem cells and dermal stem cells ***(Fortier, 2005).***

Mesenchymal stem cells are the most widely used stem cells due to their easy accessibility in tissues, such as bone marrow aspirate and fat tissue ***(Lee et al., 2004)*** and to their large capacity for *ex vivo* expansion ***(Giordano et al., 2007).*** Their immunosuppressive properties are allowing them to be used in allogenic transplantation ***(Le Blanc and Pittenger, 2005).*** They have a highly plastic differentiation potential that included not only adipogenesis, osteogenesis and chondrogenesis ***(Bruder et al., 1998),*** but also endothelial, cardiovascular ***(Gojo et al., 2003),***

neurogenic ***(Sanchez-Ramos et al., 2000)*** and neovascular differentiation ***(Kobayashi et al., 2000).***

Furthermore, MSCs can be induced to differentiate into fibroblasts, neuronal cells ***(Mimeault and Batra, 2006),*** pulmonary cells ***(Albera et al., 2005),*** pancreatic islet β cells ***(Sun et al., 2007)*** and corneal epithelial cells ***(Ma et al., 2006)*** using specific growth factors and cytokines.

It had been documented that MSCs synthesize and secrete a broad spectrum of growth factors and cytokines such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), monocyte chemoatractant protien-1(MCP-1), hepatocyte growth factor (HGF), insulin like growth factor (IGF-I) and thrombopoietin which exert diverse effects on the cells in the nearby vicinity ***(Haynesworth et al., 1996)***. These factors had been postulated to promote arteriogenesis ***(Kinnaird et al., 2004),*** support the stem cells in the crypt of the intestine ***(Leedham et al., 2005),*** protect against ischemic renal ***(Tögel et al., 2005)*** and limb tissue injury ***(Nakagami et al., 2005)***, and maintain hematopoiesis ***(Van Overstraeten-Schlögel et al., 2006)***.

Mesenchymal stem cells had been widely used in experimental studies on hearts of rats ***(Xiaohua et al., 2005; Ou et al., 2010 and Hatzistergos et al., 2010)*** and to a lesser extent in clinical studies on ischemic cardiomyopathy ***(Schuleri et al., 2009)*** to evaluate their efficacy in different diseases and conditions.

Mesenchymal stem cell administration strategies used either intramyocardial injections of cells suspended in culture medium or intravenous injections. Intramyocardial injection route was limited by low cell retention as more than 80%–90% of grafted cells die within 72 hours after injection, and high mortality rates ***(Mathieu et al., 2012).*** Intravenous delivery of MSC is a noninvasive route ***(Barbash et al., 2003).***

Mesenchymal stem cells injection was used for cardiac tissue repair and regeneration after MI ***(Segers and Lee, 2008).*** The beneficial effects of MSC injection had been partly related to their paracrine activity. MSC secrete angiogenic, antiapoptotic, and anti-inflammatory cytokines that may contribute to the recovery of cardiac function ***(Tse et al., 2003, Aggarwal and Pittenger, 2005)*** and significantly decrease fibrosis of the myocardium ***(Nagaya et al., 2005 and Li et al., 2008).*** The factors secreted by MSCs had been demonstrated to exert beneficial effects on the heart, including attenuation of ventricular wall thinning ***(Shake et al., 2002)*** and increased angiogenesis ***(Kinnaird et al., 2004 and Miyahara et al., 2006).***

Mesenchymal stem cells transplantation in most animal models of AMI generally resulted in reduced infarct size, improved left ventricular ejection fraction, increased vascular density and myocardial perfusion ***(Amado et al., 2005).***

In a clinical trial, single infusion of allogeneic MSCs in patients with AMI was documented to be safe with improved outcomes regarding cardiac arrhythmias, pulmonary function

and left ventricular function.It was examined using magnetic resonance ***(Schuleri et al., 2009).***

Cardiac resident stem cells:

It was widely accepted that the heart was not capable of regeneration and that any increase in size was due to hypertophy. However, ***Hsieh et al. (2007)*** showed that the adult heart regeneration in mice was due to the presence of a subset of progenitor cells or due to increased proliferation of resident cardiomyocytes**.**The heart contains two distinct subpopulations of endogenous stem cells: myogenic CSCs (mCSCs), which were mainly responsible for regenerating cardiomyocytes ***(Bearzi et al., 2007)*****,** and vasculogenic CSCs (vCSCs), which were more dedicated to the turnover of coronary vessels ***(Bearzi et al., 2009).***

Cardiac progenitor cells are usually defined as self-renewing, clonogenic, and multipotent cells that can differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells both in vitro and in vivo ***(Garbern and Lee, 2013).***

The site of these adult stem or progenitor cells in the wall of the heart is within a specialized microenvironment “niche". In this niche, other neighbouring cells such as fibroblasts, endothelial cells tightly regulated their functions through the direct interactions and release of specific soluble factors protecting primitive cells from harmful external stimuli ***(Mimeault and Batra, 2006).***

The cardiac stem niches were related to the epicardium mainly, dispersed through the myocardium and were most numerous at atria and the apices as they represent protected anatomical areas characterized by low hemodynamic stress ***(Leri et al., 2014).***

Stem cell population from the cardiac atrial appendage was used as a therapy for MI ***(Koninckx, 2013).***

Recently, cell transplantation of cardiac progenitor cells and MSCs forming an alloy of both of them, after infarction in mice improved myocardial repair ***(Quijada et al., 2015).***