Ultrastructural Changes in Cardiac Muscles after Long Term Administration of Amiodarone and Possible Protective Effect of Moringa oleifera Leaf Extract and Mesenchymal Bone Marrow Stem Cell on Adult Male Albino Rats

Eman E. El wakeel¹ and Amira Z. Mohamed²

1 Anatomy and Embryology Department, Faculty of Medicine, Benha University, Benha, Egypt.
2 Microbiology Department, Faculty of Science, Tanta University, Tanta, Egypt.
eman.ismail@fmed.bu.edu.eg ; elwakeelazs@gmail.com

Abstract: Background and aim: Myocardial damage is one of the most common pathological findings in the world. It is vital to locate a productive common defensive factor against the myocardial damage. The present investigation was embraced to assess cardio-protective action of aqueous extract of Moringa oleifera (M. oleifera) leaf and mesenchymal cell-on histological architecture of cardiac muscles. Stem cell therapy holds a great promise for the repair of injured tissues. Mesenchymal stem cells (MSCs) and M. oleifera leaf have the potential to present a new trend of treatment. This work targeted to ponder the impact of bone marrow-MSCs and aqueous extract of M. oleifera leaf on amiodarone-induced myocardial damage in albino rats by histological methods. Materials and methods Forty-two adult male albino rats were used. The bone marrow-MSCs source was seven rats. Thirty-five rats were divided into the following groups: negative control group included seven rats that received no treatment; vehicle control group included seven rats that received polysorbate 80; seven rats involved in group received amiodarone orally for every day for 5 weeks and also group treated with M. oleifera leaf extract included seven rats that received extract 2h before orally uptake amiodarone and stem cell-treated group included seven rats that received stem cells after amiodarone stoppage. Myocardium specimens were histologically examined. Results Amiodarone group showed disrupted myocardial architecture, lysis of myofibrils, and significant ultra-structural changes in myocardium in the form of disorganized myofibrils, swollen destructed mitochondria, SER dilatation, and cellular infiltration with mononuclear inflammatory cells. Minimal microscopic changes of the myocardium were obtained in Bone marrow-MSCs and natural extract of M. oleifera leaf treated groups with preservation of the normal structure of the cardiac myocytes. Conclusion Mesenchymal stem cells (MSCs) and M. oleifera leaf can improve the deleterious effects associated with amiodarone-induced myocardial damage.

Keywords: amiodarone, myocardial damage, mesenchymal stem cells.

1. Introduction

It was well established that long-term oral administration of amiodarone is extremely effective in the management of most supraventricular and ventricular tachyarrhythmias [1, 2]. Amiodarone was arranged as a class III antiarrhythmic medication, as it drew out the atrial and ventricular activity potential duration (APD). When the patient underwent for long term administration of the drug, these effects are potentiated [3]. Contrasted with the activity of most accessible antiarrhythmic agents, amiodarone administration orally has a negative inotropic impact of lower value and was less proarrhythmic, making it useful for use in patients with weakened ventricular function [4].

Phytopharmaceuticals were earning significance in allopathic just as alternative medicine which had less poisonous nature. Novel antioxidants may present a valuable and safe method for neutralizing a portion of the issues and supporting the body’s defense against free radicals and cardiovascular diseases. The scientific name of the therapeutic herb was Moringa oleifera Lam (Family-Moringaceae). This herb presents in Asia and tropical America and it has been known as Drum stick tree. Different pieces of this tree have been served for pharmacological activities actions. These natural herbs were useful as antispasmodic, diuretics and for healing of inflammation. The leaves of these herbs used to decrease blood pressure, level of cholesterol, and served as antulcer and for healing wounds [5,6]. Mehta et al., (2003) announced the hypolipidemic activity of its fruits [7]. Moreover, aqueous extract of this herb was reported a valuable antioxidant potential
by increasing the action of catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) [8, 9].

In the ongoing years, mesenchymal stem cells (MSCs) have broadly connected for the treatment of assortment of infections, for example, malignant tumor [10], liver, lung and brain diseases [11]. A great amount of interests were paid for treatment of cardiovascular disease. Due to secretion of various specific cytokines, MSCs have had different clinical applications in recent years; one of them is their application in treatment of myocardial infarction. The goal of this method was modulating of cytokines responses and as a result preventing the myocardial necrosis [12].

According to previous studies, transplantation of MSCs in vivo was capable of giving functional cardiomyocytes and to provide blood vessels for nourishing the forming cardiomyocytes. Although numerous examinations on the clinical application of MSC have been completed, the mechanisms of their capacities, especially the significance of soluble factors and their paracrine effects in myocardial infarction treatment have remained elusive [12].

The aim of this particular investigation was to explain the histopathological basis of amiodarone induced cardiac dysfunctions and the therapeutic effects of Moringa oleifera leaf extract and mesenchymal stem cells on histological architecture of cardiac muscles.

2. Materials and methods

1. Plant material and extraction

Dried leaves of M. oleifera were purchased from Egyptian markets. Ltd. Five hundred milligram of dried M. oleifera leaves were suspended in 5 mL of cold water at 4°C, strongly shaking for thirty second, and refrigerated for 5 min to 24 hours. Strongly shaking for many times of suspension at room temperature for a minute has been done. The water-insoluble parts of the suspension were removed by centrifuging it twice (8,000 rpm, 10 min each), and the supernatants were collected by membrane filtration. The resulting M. oleifera leaves extracts were lyophilized and stored at −20°C for future analysis. For our investigations, the concentration of M. oleifera leaves (20 mg/ml of protein) were obtained by suspended the powder into distilled water [13].

2. Drug

Amiodarone (Cardio-Mep, Mepaco-Medifood at Heliopolis) was used as 200 mg tablets that were manufactured by Arab Company of pharmaceuticals and medicinal plants (Mepaco- Medifood). Each tablet was grinded, and the required dose for each rat was weighed and dissolved in 1 ml polysorbate 80, which was the solvent for amiodarone (El-Gomhoria Company, Tanta, Egypt).

3. The experimental design

Fourth-two adult male albino rats (180 - 200 grams, 6-8 weeks old) were used in this study. The animals were purchased from the Medical Research Center, Alexandria University and were treated in accordance with the valid International Guidelines for animal experimentation. Seven rats from the animal house were used as bone marrow donors. Animal care for other 35 rats was provided by laboratory animal house unit of Faculty of Science, Tanta University. Suitable housing had been presented to rats, bred at 25 ± 1°C, with normal illumination. The same environmental conditions and free access to water and nourishment were available for all rats.

Thirty five rats were divided into the following groups:

- **Group I (control group)**: were equally subdivided into subgroups Ia, Ib, seven rats each.
  - **Subgroup Ia**: Rats represented negative control group and received no treatment.
  - **Subgroup Ib**: Rats represented vehicle control group and received 1 ml polysorbate 80 (orally through orogastric tube) daily for 5 weeks [14].

- **Group II (amiodarone-treated group)**: In this group, seven rats were received amiodarone (30 mg/kg body weight) dissolved in 1 ml polysorbate 80 (orally through orogastric tube) daily for 5 weeks [15].

- **Group III (amiodarone and M. oleifera treated group)**: It consisted of seven rats. Intraperitoneal injection of M. oleifera leaf extract at 400 mg/kg/day 2h before orally uptake amiodarone (30 mg/kg body weight) dissolved in 1ml polysorbate 80 (orally through orogastric tube) had been done to each rat daily for 5 weeks [6].

- **Group IV (amiodarone and BM-MSCs treated group)**: This group included seven rats. Each rat received amiodarone with the same dose of group II. Then, the next day after stopping of amiodarone, each rat was injected intravenously with a single dose of BM- MSCs (2×10⁵) in the tail vein. Then they were left for 4 weeks without any medication [16].

  Twenty-four hours after the last dose, the rats were anesthetized with ether inhalation and sacrificed.

4. Bone marrow-mesenchymal stem cells isolation, culture, and labeling

**Bone marrow isolation**

Seven rats were used as bone marrow donors. They were anesthetized by inhalation of light ether and then were killed. Rat’s skin of the hind limb was sterilized with betadine before incision of the skin. The femurs were carefully dissected and cleaned from the surrounding tissues, and then they were immersed in 70% alcohol for 1–2 min. finally, the bones were
processed sterile laminar flow to obtain the bone marrow [17].

**Mesenchymal stem cell culture**

These steps were done at Cell Culture Unit of Egyptian Society for Progenitor Cell Research in Cairo, Egypt. Centrifuged bone marrow for 10 min and then supernatant had been removed. Then, the pellet had been dissolved in complete media [17]. In complete media, cells were suspended and incubated in anaerobic incubator at 37°C in 5% CO₂. At the first day of the culture, the cultured cells were examined using the inverted microscope to show the presence of rounded floating cells. Periodic examination by inverted microscope was done to follow-up the growth of the cells and to exclude the presence of any infection in the culture [18]. Finally 10 ml of fresh complete media was added to the flask. The MSCs became obvious and began to acquire a spindle shape. The media were changed every three days and replaced with freshly prepared another complete media [19]. The MSCs detached using 2 ml of 0.25% trypsin-EDTA (EDTA). Cell detachment was evidenced by changing from spindle to round shape [20]. After separation, the supernatant was evacuated to leave the pellet. In pre-warmed complete media, the collected cells have been suspended [21]. The cells’ suspension was labeled with green florescent protein immunostain (GFP) according to the method described by Soleimani and Nadri., (2009) [22]. Cultured BMSCs were confirmed by morphology (fibroblast like cells) using an inverted microscope; Leica DM IL LED with camera Leica DFC295 and using fluorescence microscope for tracking of MSCs. Within thirty minute of preparation, the cells were resuspended at 2×10⁶ cells in complete medium and directly used.

5. **Biochemical estimations for the level of CK-MB and the level of cTnT**

At the end of the experiment, the following investigations were done at Biochemistry Department, Faculty of Medicine, Tanta University. Tail vein blood samples were collected after the reperfusion. Immediately, blood samples were centrifuged at 12,000 g for 10 minutes at 4°C and then the supernatants were collected for measurement at -20°C. The level of cTnT (cardiac troponin T) and activities of CK-MB (isoenzyme-creatine kinase-MB) were done utilizing a suite of commercial kits, in according to the manufacturer’s guidelines (Beyotime Institute of Biotechnology, Nanjing, China) [23].

6. **Histological and immunohistochemical examinations Histopathological studies**

Myocardial tissue after removal was immediately fixed in 10 % buffered neutral formalin solution for 36 hours. After fixation was complete, then processed gradually and embedded in paraffin to obtain paraffin blocks. Paraffin sections were cut serially of thickness 4-6 micrometers. Hematoxylin and eosin stain [24], Mallory’s trichrome stain [25] and immune stain Proliferating Cell Nuclear Antigen (PCNA) have been subjected to sections [26], for detection of the general histological structure, detection of collagen fibers and proliferating cells, respectively.

Finally, under light microscope, sections have been examined and photomicrographs were taken. Microscopic examination of the stained sections was done by Olympus Light Microscope.

Fluorescent microscope examination of the cardiac specimens of the rats that were treated with the labeled MSCs (group IV) was done to insure their incorporation into the cardiac tissue in central lab, Tanta University.

7. **Ultrastructure examinations**

For Transmission Electron Microscope (TEM) examination, small cardiac muscle specimens (1 mm³) were fixed with 2.5% buffered glutaraldehyde (pH 7.2-7.4) at 4 °C for 2 h, then added 1% osmium tetroxide as a post fixative (4 °C, 1.5 h), then the sample was immersed in serial dilution of ethanol (50, 70, 90, 95 and four times 100%, each for 15 min) for dehydration and dehydrated by acetone for 30 minutes. Finally, the fixed specimens were embedded in epoxy resin (Epoxy Embedding Medium Kit; Sigma). Semi- and ultra-thin sections were cut on LEICA Ultra microtome. Semi-thin sections (0.8 mm thick) were stained with 1% toluidine blue in 0.5% borax and observed using an Olympus light microscope. At thickness 70-90 nm, ultra-thin sections have been cut and then stained with uranyl acetate as a principal stain and lead citrate as counter stain [27] and finally the examination of ultrathin sections has been done using JEM-2100 (JEOL, Japan) transmission electron microscope at the electron microscope unit in the faculty of Agriculture, EL-Mansoura University, El-Mansoura, Egypt.

8. **Morphometrical study**

For measuring number of positive cells of Proliferating cell nuclear antigen (PCNA), image analysis system (Leica Q 500 MC program) at the Faculty of Science, Tanta University, was performed. Seven replica of different fields were examined at magnification of 400/slide in different in the control and experimental groups [28].

9. **Statistical analysis**

Statistical analysis was conducted by analysis of variance, using the mean and standard deviation by using SPSS program [29].

3. Results

Histological, histochemical and ultrastructural results

**Group I (Control group)**
The arrangement of myocardium appeared in groups as uniform bundles with anastomosing, branching sarcomeres. Their sarcoplasm was acidophilic with elongated central nuclei and nuclei of fibroblast of C.T. endomysium were flat deeply stained (Figs. 1 & 4). In Mallory trichrome (M.T.) stained sections, they showed a few amount of collagen fibers deposition between the fibers of cardiac muscle (Figs. 2 & 5). Immunohistochemical staining for PCNA of myocardium sections of the control group (subgroup Ia, Ib) revealed PCNA positive immunoreaction that appeared as brown nuclear deposits in myocardium. (Figs. 3 & 6).

Electron microscopic examination revealed nucleus with dispersed heterochromatin. H line and Z line bisected dark bands and light bands respectively. Mitochondria with abundant cristae are distributed between myofibrils. The intercalated discs were seen (Figs. 7, 8, 9).

Fig. 1: A section of rat’s myocardium of group Ia (negative control) showing branching and anastomosing cardiac muscle fibers with acidophilic sarcoplasm and central elongated vesicular nuclei (n). Flat dark nuclei of fibroblasts of C.T. endomysium were seen (F). (H & E X 1000)

Fig. 2: A section of rat’s myocardium of group Ia (negative control) showing few collagen fibers in-between the cardiac muscle fibers. (M.T x400)

Fig. 3: A Photomicrograph of PCNA immunostained myocardium section of group Ia showing positive nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

Fig. 4: A section of myocardium of group Ib showing branching and anastomosing longitudinal cardiac muscle fibers (thin arrow) with acidophilic sarcoplasm and central elongated vesicular nuclei (n). Flat dark nuclei of fibroblasts (F) of connective tissue can be seen. (H & E x 1000)
Fig. 5: A section of rat’s myocardium of group Ib showing minimal amount of collagen fibers in-between the cardiac muscle fibers. (M.T x400)

Fig. 6: A Photomicrograph of PCNA immunostained myocardium section of group Ib showing positive nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

Fig. 7: An electron micrograph of part of rat’s myocyte of group Ia showing part of nucleus with dispersed heterochromatin (N). Cytoplasm with myofibrils showing alternates dark bands (A) which bisected by (H) zone & light bands (I) which bisected by Z line. Mitochondria (M) with abundant cristae are distributed between myofibrils. Notice: the intercalated discs (arrow). (TEM x8000)

Fig. 8: An electron micrograph of cardiac muscle fibers of group Ib showing part of oval euchromatic nucleus with regular outline (N). Most probably normal architecture of myofibrils showing alternate dark bands (A) which bisected by (H) zone (arrow head) & light bands (I) which bisected by Z line. Normal mitochondria (M) which are distributed between myofibrils. (TEM x8000)

Fig. 9: An electron micrograph of part of rat’s myocyte of group Ib showing more or less normal continuous intact intercalated disc (arrow). Cytoplasm with myofibrils showing alternates dark bands (A) which bisected by (H) zone & light bands (I) which bisected by Z line. Most probably normal mitochondria (M) between myofibrils. (TEM x17500)
**Group II (amiodarone-treated group):**

Light microscopic examination revealed marked histological changes in the cardiac muscle fibers. Wide separation of cardiac muscle fibers could be seen in the myocardium and also branching longitudinal and transverse myocytes had dilated intercellular spaces between them. Focal extensive interstitial mononuclear cellular infiltration and dilated blood capillaries with hemorrhage in between myocardial fibers were seen (Figs. 10 & 11). Some myocardial fibers were severely affected; they showed intensely infiltration with inflammatory cells and collagen fibers. Focal areas of fragmentation and cytolysis of myofibers with loss of the cross striations in their cytoplasm were observed (Fig. 12). In Mallory trichrome stained sections, they showed massive amount of collagen fibers deposition between the cardiac muscle fibers (Fig. 13). While immunohistochemical staining for PCNA of myocardium sections of this group showed negative reaction (Fig. 14). Morphometric analysis revealed significant decrease in mean number of PCNA positive cells in amiodarone treated group (Table 1).

Electron microscopic examination revealed fragmentation and lysis of myofilaments of myocytes sarcoplasm with focal loss of the sarcomeres normal arrangement. Variable shape, size and irregular arrangement of mitochondria with dense homogenous mitochondrial matrix, in addition to dilatation of smooth sarcoplasmic reticulum were observed. Dilated cisternae of SER & T tubules were noticed and also dilated congested blood vessel were observed (Figs. 15 & 16).

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**Fig. 10:** A section of rat’s myocardium of group II showing interstitial mononuclear cellular infiltration (arrow) and collagen fibers (asteric). Some myocytes with deeply eosinophilic cytoplasm (thin arrow) and areas of hemorrhage and extravasated blood (arrow head) were observed. (H & E X 1000)

**Fig. 11:** A section of rat’s myocardium of group II showing transverse cardiac muscle fibers with eosinophilic sarcoplasm and central pale pyknotic nuclei (arrowhead). Focal lytic area of sarcomere (arrow). Infiltration with inflammatory cells (thin arrow) and collagen (asteric) could be seen. (H & E X 1000)

**Fig. 12:** A section of rat’s myocardium of group II showing dilated spaces between longitudinal cardiac muscle fibers (thin arrow). Focal areas of destruction and cytolysis of myocytes (circle) were observed. Numerous myocytes with deeply eosinophilic cytoplasm (thick arrow) and dilated blood capillaries (arrow head) were seen. (H & E X 1000)

**Fig. 13:** A section of rat’s myocardium of group II showing massive amount of collagen fibers in- between the cardiac muscle fibers. (M.T x400)
Fig. 14: A photomicrograph of PCNA immune-stained myocardium section of group II showing negative nuclear immunoreaction in vesicular nuclei. (PCNA x400)

Fig. 15: An electron micrograph of cardiac muscle fibers of group II showing infiltration with collagen (C), sarcoplasmic vacuolation (V), rarefied sarcoplasm and dilated SER (arrow) and loss of regular arrangement of myofibrils (F) and degenerated mitochondria (M). Notice an interstitial macrophage with secondary lysosome in its cytoplasm (L). (TEMx 8000)

Fig. 16: An electron micrograph of cardiac muscle fibers of group II fragmentation (arrows) and irregular arrangement of myofilaments with partial loss of the normal pattern of sarcomeres (circle). Distorted intercalated disc (thin arrow) was observed. Notice dilated sarcolemma (arrow head), rarified sarcoplasm (asteric), dense mitochondria (M) with irregular shapes and arrangement and congested blood vessel with RBCs. (TEMx 8000)

Group III (amiodarone and M. oleifera - treated group)
Light microscopic examination revealed appearance more or less similar to control group with minimal changes in the myocardium architecture with hardly detected any amount of collagen fibers in C.T. interstitium (Figs. 17 and 18). Immunostaining reaction for PCNA of myocardium sections of the group III showed moderate PCNA positive immunoreaction that appeared as brown nuclear deposits in myocardium (Fig. 19). Morphometric analysis revealed mild decrease in mean number of PCNA positive cells in group III in comparison to control group (Table 1). Most probably normal myofibrils could be observed during electron microscopic examination compared to control group. But cytoplasmic rarefaction and degeneration of mitochondria still observed (Figs. 20 & 21).
Fig. 17: A section of rat’s myocardium of group III showing more or less normal myocardium architecture with minimal lysis in the myofibril (arrow head). (H & E X 1000)

Fig. 18: A section of rat’s myocardium of group III showing minimal amount of collagen fibers in-between the cardiac muscle fibers. (M.T x400)

Fig. 19: A photomicrograph of PCNA immunostained myocardium section of group III showing moderate positive nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

Fig. 20: An electron micrograph of cardiac muscle fibers of group III showing part of oval euchromatic nucleus with regular outline (N). Most probably normal architecture of myofibrils. (TEM x17500)

Fig. 21: An electron micrograph of part of rat’s myocyte of group III showing intact continuous intercalated disc (arrow). Mild rarefication of cytoplasm between myofibrils (arrow head) and swollen degenerated mitochondria (circle).

**Group IV (amiodarone and BM-MSCs treated group)**

Labeling using green fluorescent protein immune stain (Sigma Aldrich, USA) were performed to bone marrow derived mesenchymal stem cells according to the manufacturer’s protocol. Culture on day fifteen showing adherent spindle shaped cells with multiple interfacing cytoplasmic processes (Fig.22).
Fluorescent microscope examination of the cardiac muscle specimens of the rats that were treated with the labeled BM-MSCs (group IV) was done to insure their incorporation into the myocardium tissue (Fig. 23).

Light microscopic examination revealed most probably normal longitudinal cardiac muscle fibers similar to control group (Fig. 24). In Mallory trichrome stained sections, collagen fibers deposition could be observed between the cardiac muscle fibers with a few amount (Fig. 25). Immunostaining for PCNA of myocardium sections showed PCNA strong positive immunoreaction. (Fig. 26). Morphometric analysis revealed that the mean number of PCNA positive cells in group IV was very close to that of the control group (Table 1).

Electron microscopic examination showed the ultra-structural of myofibril retained their normal appearance. Sarcoplasm showed organized arrangement of dark bands and light bands. Mitochondria (M) with abundant cristae were distributed between myofibrils. (Figs. 27, 28).

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**Fig. 22:** An inverted microscope micrograph on day 15 from a primary culture of BM-MSCs showing many spindle shaped stem cells. (**Inverted microscope x200**)

**Fig. 23:** A fluorescent microscope micrograph showing fluorescence emission intensity from a thin section of cardiac muscle labeled with multiple fluorophores scattered in the myocardium tissue of group IV. (**Fluorescent microscope x400**)

**Fig. 24:** A section of rat’s myocardium of group IV showing most probably normal branching cardiac muscle fibers with acidophilic sarcoplasm and central elongated vesicular nuclei (n). Flat dark nuclei of fibroblasts of C.T. endomysium were seen (F). (**H & E x 1000**)

**Fig. 25:** A section of rat’s myocardium of group IV showing few amount of collagen fibers in-between the cardiac muscle fibers. (**M.T x400**)

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Fig. 26: A photomicrograph of PCNA immunostained myocardium section of group IV showing strong positive nuclear immunoreaction in vesicular nuclei (arrow). (PCNA x400)

Fig. 27: An electron micrograph of part of rat’s myocyte of group IV showing nucleus with dispersed heterochromatin (N). Cytoplasm with myofibrils showing alternates dark bands (A) which bisected by (H) zone & light bands (I) which bisected by Z line. Mitochondria (M) with abundant cristae were distributed between myofibrils. (TEM x8000)

Fig. 28: An electron micrograph of part of rat’s myocyte of group IV showing intact intercalated disc (arrow). Sarcoplasm with myofibrils revealed alternates dark bands (A) which bisected by (H) zone & light bands which bisected by Z line. Most probably normal mitochondria (M) between myofibrils. (TEM x17500)

Table 1: Morphometric analysis of the cardiac muscle in different groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean number of PCNA positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102.6 ± 2.47</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>37.8* ± 1.37</td>
</tr>
<tr>
<td>Amiodarone + Morenga</td>
<td>80.8 ± 3.29</td>
</tr>
<tr>
<td>Amiodarone + BM-MSCs</td>
<td>94.7 ± 1.55</td>
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</tbody>
</table>

Data are expressed as means ± S.E.M. *Significant (p< 0.05) as compared to control group

Cardiac markers

As shown in Table 2, treatment of rats with amiodarone significantly increased the levels of isoenzyme-creatine kinase-MB (CK-MB) and cardiac troponin T (cTnT) in the serum as compared to control animals. While no significant changes were observed in rats treated with Morenga and BM-MSCs. BM-MSCs treated group showed improvement in the level of cardiac parameters and retained to their normal levels (Figs. 29, 30).

Table 2: Effect of amiodarone on the levels of cTnT and CK-MB.

<table>
<thead>
<tr>
<th>Groups</th>
<th>cTnT (ng/ml)</th>
<th>CK-MB (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.54 ± 0.02</td>
<td>103.67 ± 1.18</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>1.92* ± 0.14</td>
<td>147.36* ± 1.46</td>
</tr>
<tr>
<td>Amiodarone + Morenga</td>
<td>1.63 ± 0.06</td>
<td>125.28 ± 2.31</td>
</tr>
<tr>
<td>Amiodarone + BM-MSCs</td>
<td>0.59 ± 0.06</td>
<td>108.13 ± 1.17</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E.M. Significance was determined by One-Way ANOVA, P < 0.001.
4. Discussion

In cardiac patients, amiodarone represented anti-arrhythmic drug and effective in suppressing arrhythmias without increasing sudden cardiac death [30]. However, the chronic administration of amiodarone for long term revealed adverse impact on myocardium and required further investigation, as experimental studies to date have yielded conflicting results [31]. In addition, the chronic administration of amiodarone resulted on myocardial fibrosis as a side effect of this drug remains unclear; profibrotic effects in the lung parenchyma were observed in further investigation by giving long term therapy of amiodarone [32,33]. So the effects of long-term amiodarone therapy on myocardial fibrosis and myocardial damage were targeted in our investigation. Many side effects such as myocardial fibrosis and other abnormalities changes of cardiac muscle can be observed by administration of the amiodarone doses for long time. These results were in line with Anastasia et al. (2016) [34].

In this study, light microscopic examination in amiodarone treated group revealed sarcoplasmic vacuolation of some myofibers and disorganization of sarcomeric structure, in addition to lysis of some sarcomeres. These findings were in agreement with Liu et al. (2002) [35] and Pathan et al. (2010) [36]. Mallory trichrome stained sections confirmed myocardium fibrosis by excessive deposition of collagen fibers in amiodarone treated group in between myofibril, some myofibrils with pyknotic nuclei, dark cytoplasm and focal areas of lytic fibers were seen. These results were confirmed with an apparent negative PCNA immunoreactivity in most of nuclei in muscle fibers in amiodarone treated animals. This was confirmed by results of several investigators [37, 38].

In the present study, amiodarone treated group showed rupture of wall of blood vessels with area of hemorrhage between muscle fibers. Marked vascular dilatation was noticed; this coincided with the work of other authors who added that increased accumulated blood cells between the damaged cardiac myocytes were noticed [39]. After amiodarone treatment, inflammatory cellular infiltration was observed. These findings were in agreement with other investigation [40]. The previous light microscopic changes of amiodarone group were confirmed by electron microscopy which showed disarrangement of sarcomeres, degeneration of myofibrils with thinning of myofibrils bundle and also widening in cisterne of smooth endoplasmic reticulum and T tubules. There was irregular arrangement of mitochondria with dense homogenous mitochondrial matrix. Some fibers however, preserved normal fine structure. This result was in agreement with other researchers. The toxic effect of amiodarone was refereed to oxidative stress and the release of oxygen free radicals. The oxidative stress due to an increased amount of oxygen free radicals could cause oxidative damage to the mitochondria and release cytochrome C from mitochondrial intermembrane space, resulting in apoptosis [41]. Also with mitochondrial changes, there is decreased production of adenosine triphosphate which intern leading to intercalated disc dissociation, disintegration of filaments and sarcomere irregularities [42].

A huge numbers of researches has been recorded the valuable importance of *Moringa oleifera* leaf. It is however important to note that there is the need for more specific reports, especially considering the scope of the research activities leading to the presented results. As it will be essential to use the plant’s products as standard nutritional supplements. In this study, the histological results of *Moringa oleifera* treated group showed light microscopic results more or less similar to control with mild lysis of myofibril and minimal infiltration with collagen fibers. It revealed moderate positive reaction of PCNA. This was in agreement with the previous findings of other investigators [43, 44]. Electron microscopic examination revealed mild rarified sarcoplasm with partial lysis of cristae of mitochondria while in general it has been observed the improvement in the
sarcomeric architecture in general. In the present study, *M. oleifera* leaf extract had its improvement activity through various mechanisms including its antioxidants and antiapoptotic effects to reduce the effects of free radicals, altered profibrotic gene expression, free radical scavenging. These results were also similar to the observation of some authors [45, 46]. Moreover, aqueous extract of this herb was reported a valuable antioxidant potential by increasing the action of catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) [8, 9].

One of the most promising therapeutic strategies was myocardial regeneration for heart failure patients. In several experimental investigations, different types of stem cell can be differentiate into myocardial cells and other tissues and these studies have been demonstrated that BM-MSCs were necessary for regeneration the damaged myocardium, while studies in experimental animals suggested that muscle (myoblast), bone marrow (mesenchymal or hematopoietic progenitors) and even heart cells can help to improve heart contractility in vivo [47,48]. In the present study, BM-MSCs treated group showed improvement in the whole architecture of myocardium with intact intercalated disc with normal appearance of myofibril and other organelles. These data were in agreement with Maximilian. (2019) [48]. Immunostaining for PCNA of myocardium showed PCNA strong positive immunoreaction compared to amiodarone treated group due to the differentiation and proliferation of BM-MSCs in the sarcomeric structure. These results were in line with other investigation [49]. The use of Mesenchymal bone marrow stem cells resulting in modulation of cytokines responses and as a result preventing the myocardial necrosis [12].

Also the levels of cardiac enzymes showed significant increase in levels of isoenzyme- creatine kinase-MB (CK-MB) and cardiac troponin T (cTnT) in amiodarone treated group while the levels of these enzymes decreased in the *M. oleifera* treated group and retained to its normal level in the BM-MSCs treated group due to the differentiation of BM-MSCs. It was confirmed in other study [50].

5. Conclusion

In conclusion, from this investigation it is cleared that *Moringa oleifera* leaf extract and mesenchymal bone marrow stem cells treatment, after and in concurrent with amiodarone, markedly attenuate amiodarone induced cardiac changes and myocardial damage. It is recommended to use *M. oleifera* leaf extract in concurrent with amiodarone. So, the combined treatment of *M. oleifera* leaf extract and amiodarone make an effective and safe therapeutic strategy and mesenchymal bone marrow stem cells are effective in improvement of any damage in cardiac muscle.

Conflict of interest

There are no conflicts of interest.

Corresponding author:

Dr. Eman El wakeel

TQM, MD, DHPE, Anatomy and Embryology Department, Benha faculty of medicine, Benha University, Egypt,

E-mails: eman.ismail@fmed.bu.edu.eg elwakeelazs@gmail.com

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