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# Down-regulation of CD68 after simvastatin treatment of isoproterenol-induced myocardial infarction in rats

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ABSTRACT

Isoproterenol (ISO) induces myocardial injuries in the form of ischemia and infarction (MI). Simvastatin (SIM) is a lipid-soluble inhibitor of hydroxy-3-methylglutaryl coenzyme A reductase with multiple reported therapeutic benefits. The present study was designed to evaluate the effect of pre-treatment with SIM on ISO-induced cardiac infarction in rats. Forty-eight rats were divided into four groups. Group I (control) received normal saline. Group II (SIM) received SIM (10 mg/kg body weight, orally by gavage) for 30 days. Group III (ISO) received ISO (5 mg/kg) intraperitoneally for 7 days to induce cardiac injury. Group IV (ISO/SIM); received SIM (10 mg/kg body weight, orally by gavage) for 30 days and in the last 7 days they received ISO (5 mg/kg) intraperitoneally. Serological analysis for detection of cardiac injury markers (troponin-T and creatine phosphokinase-MB "CPK-MB") and inflammatory markers (IL-6 and TNF-α) was done. Cardiac tissues were processed for histological examination (H&E and Masson's trichrome) and for the immunohistological quantitative analysis of CD68. Administration of ISO induced an increase in heart weight to body weight (HW/BW) ratio and elevation of systolic and diastolic blood pressure. Serological analysis revealed an increase of interleukin-6, troponin-T, (CPK-MB), and tumor necrosis factor-α (TNF-α). Histopathological examination of heart tissue revealed thickening of the left ventricle and inter-ventricular septum, large focal areas of sub-endocardial degeneration, mononuclear cellular infiltrations, and massive interstitial fibrosis. In addition, ISO-treated rats exhibited significant up-regulation of CD68. Pre-treatment with SIM significantly attenuated ISO-induced cardiac hypertrophy and necrosis, alleviated the elevated biochemical parameters and CD68 expression, and improved the heart histopathological changes. This study provides evidence that SIM minimizes the ischemic effect of ISO on the heart of rats through inhibition of inflammatory cellular infiltration, especially macrophages, as confirmed by down-regulation of CD68.



## 1. Introduction

Cardiac hypertrophy and ischemia represent major causes of morbidity and mortality in the world (Lorell and Carabello, 2000). Prolongation and prevalence of hypertension lead to congestive heart failure (CHF) and sudden death (Yung et al., 2004). Heart failure (HF) is a major health burden accounting for approximately 25% of all deaths in developing countries and patients with HF have a 50% mortality rate within 4 years (Lopez, 1992; Dickstein et al., 2008). Also, cardiomyocytes hypertrophy has been reported to often occur after myocardial infarction (MI) as an adaptive response (Pfeffer and Braunwald, 1990; Richey and Brown, 1998). MI is a complication of cardiovascular disease and its prevalence is growing rapidly in developing countries probably due to the acquisition of a western lifestyle (Steyn et al., 2005).

Isoproterenol (ISO), a synthetic catecholamine and beta-adrenergic agonist, has been found to produce myocardial hypertrophy and MI due to generation of highly cytotoxic free radicals, causing cardiac dysfunctions, increased lipid peroxidation, altered activities of cardiac enzymes and antioxidants, resulting in infarct-like necrosis of the heart muscle (Rathore et al., 1998). The

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pathophysiological and morphological alterations of the myocardium following ISO administration have been observed to be similar to those taking place in human MI (Wexler, 1978).

Statins, hydroxyl-methylglutaryl coenzyme A reductase inhibitors, are widely prescribed cholesterol-lowering agents. They have been reported to exert multiple protective effects on the cardiovascular system decreasing the incidence of myocardial infarction and ischemic stroke, independent of their classical functions on lipoproteins (Kapur and Musunuru, 2008; Koh et al., 2011). Several studies have demonstrated that early and chronic pre-treatment with statins can improve myocardial perfusion and decrease the sizes of no-reflow and infarction areas after ischemic reperfusion. These effects were mainly through the inhibition of myocardial inflammation and apoptosis and the improvement of endothelial function (Iwakura et al., 2006; Zhao et al., 2006; Manickavasagam et al., 2007; Merla et al., 2007). Simvastatin (SIM), a member of statins, has been recognized to have antioxidant effects and is an effective agent for preventing the development of cardiac hypertrophy (Takemoto et al., 2001).

Macrophages belong to the mononuclear phagocyte system (Sunderkotter et al., 1994) and are derived from CD34+ bone marrow progenitors (Lewis and Pollard, 2006). Monocytes enter the blood stream and extravasate into injured tissues in response to chemotactic signals (Sunderkotter et al., 1994; Crowther et al., 2001; Lewis and Pollard, 2006). Macrophage activation up regulates phagocytic, chemotactic, secretory, and angiogenic functions (Porcheray et al., 2005). While neutrophil response is associated with the early, acute inflammatory events, macrophage cells continue to be present during the proliferation phase and initial deposition of collagen in a model of right ventricular damage (Watts et al., 2008). Therefore, the current study was designed to investigate the protective role of SIM against ISO-induced cardiac hypertrophy and infarction in rats focusing on its influence on monocytes/macrophages.

## 2. Materials and methods

### 2.1. Experimental animals

Forty-eight adult male Wistar albino rats weighing 190 ± 20 g were supplied by the Experimental Animal Centre at the College of Medicine, King Saud University, Riyadh, Saudi Arabia. The rats were maintained in controlled environment (21-23°C and 40-50% humidity) on a 12-hour light/dark cycle with standard laboratory food and water ad libitum. Rats were kept under observation for one week before the onset of the experiment for acclimatization and to exclude infection. All the experimental procedures were conducted according to the Guidelines for the Care and Use of Laboratory Animals of the College of Medicine Research Center (CMRC) at King Saud University and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH).

### 2.2. Chemicals

Mouse monoclonal anti-CD68 antibody [ED1] was purchased from abcam (Cambridge, USA). ISO and SIM were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial supplies.

### 2.3. Experimental design

Forty-eight Rats were randomly allocated into four groups (twelve in each) as follows:

- Group I (Control group): received normal saline, orally, by gavage, for 30 days and intraperitoneally for the last 7 days.
- Group II (SIM group): received SIM (10 mg/kg/day) (Chen et al., 2015) dissolved in saline, orally, by gavage, for 30 days and intraperitoneal saline for the last 7 days.
- Group III (ISO group): received normal saline, orally, by gavage, for 30 days and daily ISO (5 mg/kg) intraperitoneally (Wan et al., 2011) for the last 7 days.
- Group IV (ISO/SIM group): received SIM (10 mg/kg) dissolved in saline, orally, by gavage, for 30 days and daily ISO (5 mg/kg) intraperitoneally for the last 7 days.

The doses were adjusted consistently as indicated by any change in body weight (BW) to maintain comparable dosage over the study period. At the end of the experiment, blood samples were collected, from retro-orbital plexus of the animals, and centrifuged to separate serum. Sera were then kept at -20°C for subsequent biochemical assays. After that, the rats were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally) and hearts were immediately excised, rinsed in ice-cold normal saline, and then fixed in 10% buffered formalin for processing.

### 2.4. Assessment of body weight, heart weight, and blood pressure

The final BW along with the heart weight (HW) was recorded, and the heart-to-body weight (HW/BW) ratio was calculated and used to estimate the degree of cardiac hypertrophy. The arterial blood pressure (BP) was measured using the tail cuff method (UGO BASILE, Italy) before and after the administration of ISO with minimal stress and restraint. Systolic and diastolic BP was measured before and at the end of treatment period.

### 2.5. Serum biochemical analysis

Determination of serum levels of troponin-T and creatine phosphokinase-MB (CPK-MB): Serum levels

of troponin-T were measured using a Siemens Dimension Xpand Plus instrument (IL, USA). Serum levels of CPK-MB isoenzyme were determined with an auto-analyser (ILab-300, bioMérieux Diagnostics, Milan, Italy). Both troponin-T and CPK-MB are cardiac injury markers.

Determination of serum levels of tumor necrosis factor-alpha (TNF-α): The serum concentration of TNF-α (an inflammatory cytokine) was measured using enzyme-linked immunosorbent assay (ELISA) kits following the instructions supplied by the manufacturer (DuoSet Kits, R&D Systems, Minneapolis, MN, USA).

Determination of serum levels of interleukin-6 (IL-6): Serum levels of IL-6 were measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay, R&D Systems, Minneapolis, MN, USA) (Kaden, 2007).

### 2.6. Histopathological analysis

The excised heart tissues were fixed in 10% buffered formalin at 4°C for 48 hours and processed to prepare transverse, mid-ventricular sections. The fixed tissues were embedded in in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (H&E) and Masson's trichrome stains. The sections were then examined and photographed using Olympus Bx51 light microscope equipped with a DP70 camera (Olympus, Tokyo, Japan).

### 2.7. Immunohistochemical analysis

Immunostaining of the heart sections for CD68 to visualize monocytes/macrophages was performed using streptavidin-biotinylated horseradish peroxidase method (Novalink Max Polymer detection system; Novocastra Laboratories, Newcastle, UK). The procedure involved the following steps: endogenous peroxidase activity was inhibited by 3% $H_2O_2$ in distilled water for 5 minutes, and then the sections were washed twice in Tris-buffered saline (TBS, pH 7.6) (Sigma-Aldrich) for 10 minutes. Nonspecific binding of antibodies was blocked by incubation with protein block for 10 minutes (Novocastra). Sections were incubated with mouse monoclonal anti-CD68 antibody [ED1] (ab31630), diluted 1:100 for 1 hour at room temperature. Sections were washed three times in TBS and then incubated with biotinylated IgG (Novocastra) for 30 minutes, followed by washing in TBS and incubation with Novolink polymer (Novocastra) for 30 minutes. Peroxidase was detected with working solution of diaminobenzedine (DAB) chromogen (Novocastra) for 10 minutes. Sections were then washed in distilled water for 10 minutes. Counterstaining was carried out with Mayer's hematoxylin to stain the nuclei, and sections were mounted in DPX (Dysterene, Plasticizer, Xylene). For negative control sections, the same procedure was followed with omission of incubation in the primary antibody.

### 2.8. Image analysis

High-resolution whole-slide digital scans of all stained sections were created with a ScanScope scanner (Aperio Technologies, Inc.). The digital slide images were then viewed and analyzed using the viewing and image analysis tools of Aperio's ImageScope software (Aperio Technologies, Inc.). Five areas, each with the fixed size of 0.25 $mm^2$, were randomly selected per section. To quantify the immunopositive reaction, the color deconvolution (color separation) algorithm (Aperio Technologies, Inc.) was set up (by color calibration) to detect and quantify only the brown color of DAB positive staining. The algorithm was then run on the selected area to measure the percentage of immunopositive reaction relative to the total analysis area.

The thickness of the wall of the left ventricle was measured on the digital scans of H&E-stained slides, using the linear measurement tool of Aperio's Image Scope software (Aperio technologies, Inc.). To minimize error, thickness was measured at five randomly chosen points per heart section and averages were calculated.

The extent of fibrosis was measured on the digital scans of Masson's trichrome-stained slides. Five areas, each with the fixed size of 0.25 $mm^2$, were randomly selected per section. Color deconvolution (color separation) algorithm (Aperio Technologies, Inc.) was then applied so as to select and measure the area of only the green color of fibrous tissue (as stained by Masson's trichrome) and calculate its area percentage relative to the total area of analysis. All image analysis output results were finally exported to Excel sheets and subjected to statistical analysis.

### 2.9. Statistical analysis

Data collected were subjected to statistical analysis using IBM SPSS Statistics version 22 software. The homogeneity of variance was first checked with Levene test. Analysis of variance (ANOVA) was used for an overall comparison between the groups followed by Bonferroni test (when the homogeneity of variance assumption was met) or by Games-Howell test (when the homogeneity of variance assumption was not met) as post-hoc tests for pairwise comparisons. Differences were considered significant when P was less than or equal to 0.05.

## 3. Results

### 3.1. BW, HW, HW/BW ratio and BP

Body weight of the control (group I) and treated groups (groups, II, III & IV) showed non-significant ($P>0.05$) changes throughout the experiment. On the other hand, ISO-injected rats (group III) exhibited significant ($P<0.01$) increase in HW and HW/BW ratio when compared with the control rats. Treatment of the ISO-injected rats with SIM (group

IV) significantly (P<0.01) ameliorated both the HW and HW/BW ratio as compared to ISO-injected rats (group III). Treatment with SIM alone (group II) produced a non-significant (P>0.05) effect on the mentioned parameters when compared with control rats (control group). Similarly, ISO administration (group III) produced a significant (P<0.001) elevation in systolic and diastolic BP. Oral administration of SIM (group IV) significantly (P<0.01) alleviated the altered BP levels when compared with the ISO-treated rats (group III) (Table 1).

**Table 1:** Mean BW, HW, HW/BW ratio and BP in the studied groups.

| Parameter | Control (Group I) | SIM (Group II) | ISO (Group III) | ISO/SIM (Group IV) |
|---|---|---|---|---|
| BW (g) | 230.10±7.82 | 229.80±7.13 | 240.71±9.50 | 234.22±6.86 |
| HW (g) | 0.86±0.02 | 0.84±0.02 | 1.40±0.05** | 1.12±0.02## |
| HW/BW ratio | 0.0037±0.00 | 0.0036±0.00 | 0.0058±0.00** | 0.0047±0.00## |
| SBP (mmHg) | 136.17±10.61 | 147.62±11.05 | 285.50±7.58*** | 229.63±15.60## |
| DBP (mmHg) | 79.52±3.49 | 100.64±2.75* | 201.24±11.7*** | 111.81±6.52## |

Data are mean ± SE (N=7). *$P$, 0.05; **$P$, 0.01; ***$P$, 0.001 vs control. ##$P$, 0.01 vs ISO group

### 3.2. Left ventricular wall thickness

H&E stained heart sections showed significant increase of left ventricular wall thickness of ISO-injected rats (group III) compared to the other three groups (groups I, II, and IV), and by SIM treatment (group IV) the thickness significantly decreased compared to ISO-injected rats (group III) (Table 1).

**Table 2:** Mean left ventricular wall thickness (μm) in the studied groups.

| | Group I (Control) | Group II (SIM) | Group III (ISO) | Group IV (ISO/SIM) |
|---|---|---|---|---|
| Mean ± SE | 3. 346 ± 0.082 | 3.481 ± 0.019 | 5.142 ± 0.161 | 4. 022 ± 0.143 |
| *P1* | | 1.000 | 0.000* | 0.000* |
| *P2* | 1.000 | | 0.000* | 0.000* |
| *P3* | 0.000* | 0.000* | | 0.000* |

SE, standard error; *P1*, versus group I; *P2*, versus group II; *P3*, versus group III; * significant difference ($P \leq 0.05$)

### 3.3. Serum biochemical analysis

Serum levels of CPK-MB and troponin-T (Tables 3 and 4): Serum CPK-MB (Table 3) was significantly elevated in ISO-injected rats (group III) compared to control group (group I), but significantly decreased after treatment with SIM (group IV). Treatment with SIM alone (group II) produced no significant change compared to control rats (group I). Similarly, troponin-T (Table 4) was significantly elevated in ISO-injected rats (group III) compared to control rats (group I), but significantly decreased by SIM treatment (group IV). Treatment with SIM alone (group II) produced no significant change compared to control rats (group I).

Serum levels of TNF-α and IL-6 (Tables 5 and 6): Serum inflammatory markers (TNF-α and IL-6) were significantly elevated in ISO-injected rats (group III) as compared to the other three groups (groups I, II, and IV), but significantly decreased by SIM treatment (group IV).

**Table 3:** Mean serum CPK-MP levels (U/L) in the studied groups.

| | Group (Control) | Group II (SIM) | Group III (ISO) | Group IV (ISO/SIM) |
|---|---|---|---|---|
| Mean ± SE | 31.280 ± 0.306 | 31.652 ± 0.456 | 182.646 ± 1.617 | 113.498 ± 1.368 |
| *P1* | | 1.000 | 0.000* | 0.000* |
| *P2* | 1.000 | | 0.000* | 0.000* |
| *P3* | 0.000* | 0.000* | | 0.000* |

SE, standard error; *P1*, versus group I; *P2*, versus group II; *P3*, versus group III;
* significant difference ($P \leq 0.05$)

**Table 4:** Mean serum troponin-T levels (pg/ml) in the studied groups.

| | Group I (Control) | Group II (SIM) | Group III (ISO) | Group IV (ISO/SIM) |
|---|---|---|---|---|
| Mean ± SE | 6.538 ± 1.469 | 6.330 ± 0.499 | 48.834 ± 0.966 | 26.099 ± 0.711 |
| *P1* | | 1.000 | 0.000* | 0.000* |
| *P2* | 1.000 | | 0.000* | 0.000* |
| *P3* | 0.000* | 0.000* | | 0.000* |

SE, standard error; *P1*, versus group I; *P2*, versus group II; *P3*, versus group III;
* significant difference ($P \leq 0.05$)

### 3.4. Histopathological analysis

Light microscopic examination of H&E-stained heart tissues from control (group I) and SIM-treated (group II) rats showed normal microscopic features of cardiomyocytes and interstitial connective tissue without any evidence of necrosis or inflammation (Fig. 1 A and B). In contrast, H&E-stained sections of the heart from ISO-injected rats (group III) showed wide sub-endocardial necrotic patches along with

interstitial edema. In addition, focal areas of confluent necrosis of cardiac muscle fibers, inflammatory cellular infiltration, and vacuolar degeneration were also observed (Fig. 1C).

**Table 5:** Mean serum TNF-α levels (pg/ml) in the studied groups

| | Group I (Control) | Group II (SIM) | Group III (ISO) | Group IV (ISO/SIM) |
|---|---|---|---|---|
| Mean ± SE | 7.164 ± 0.493 | 6.686 ± 0.393 | 34.954 ± 1.226 | 15.350 ± 0.940 |
| *P1* | | 1.000 | 0.000* | 0.000* |
| *P2* | 1.000 | | 0.000* | 0.000* |
| *P3* | 0.000* | 0.000* | | 0.000* |

SE, standard error; *P1*, versus group I; *P2*, versus group II; *P3*, versus group III;
* significant difference ($P \leq 0.05$)

**Table 6:** Mean serum IL-6 levels (pg/ml) in the studied groups

| | Group I (Control) | Group II (SIM) | Group III (ISO) | Group IV (ISO/SIM) |
|---|---|---|---|---|
| Mean ± SE | 8.028 ± 0.320 | 7.634 ± 0.319 | 33.386 ± 3.061 | 13.664 ± 0.652 |
| *P1* | | 1.000 | 0.000* | 0.000* |
| *P2* | 1.000 | | 0.000* | 0.000* |
| *P3* | 0.000* | 0.000* | | 0.000* |

SE, standard error; *P1*, versus group I; *P2*, versus group II; *P3*, versus group III;
* significant difference ($P \leq 0.05$)

In ISO/SIM group (group IV) there were only and few, small focal areas of necrosis with mild interstitial edema and less inflammatory cellular infiltration (Fig. 1D) compared to ISO group (III).

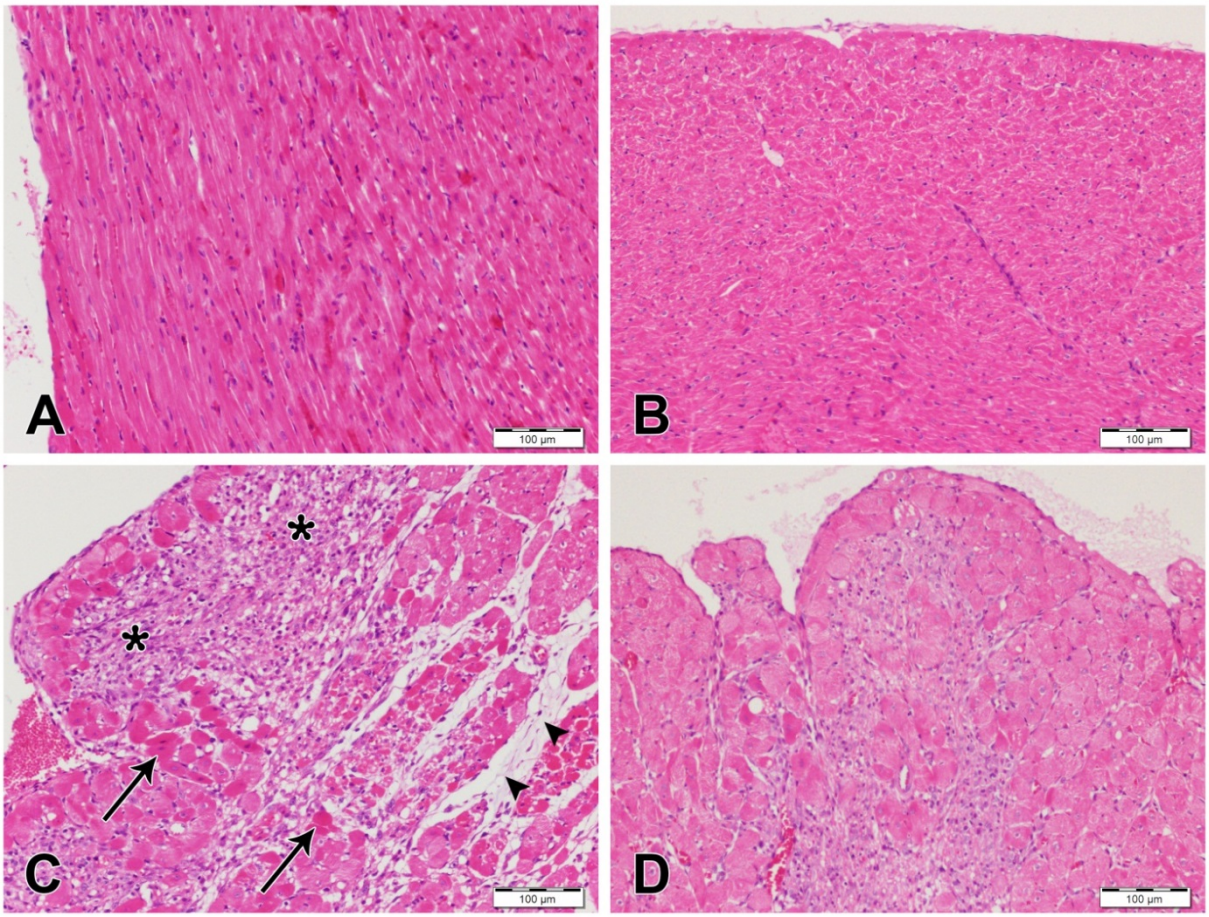


**Fig. 1:** Heart sections stained with H&E. (A) Control group, and (B) SIM group showing normal histological architecture. (C) ISO group showing patches of marked sub-endocardial myocardial cellular degeneration and necrosis (arrows), areas of complete loss of cardiomyocytes and replacement with fibrous tissue infiltrated with inflammatory cells (asterisks), and areas of interstitial edema (arrowheads) (D) ISO/SIM group showing marked decrease of myocardial degeneration, interstitial edema and inflammatory cellular infiltration. [Scale bars = 100 µm]

Masson's trichrome stained heart sections from control (group I) and SIM (group II) rats showed normal sparse distribution of fibrous tissue, restricted to the endomysium and wall of blood vessels (Fig. 2 A and B). Heart sections from ISO-injected rats (group III) showed marked increase of the sub-endocardial fibrous tissue deposition, partially replacing necrotic myocardium (Fig. 2C), while SIM treatment (group IV) significantly reduced this interstitial fibrosis (Fig. 2D, Table 7).

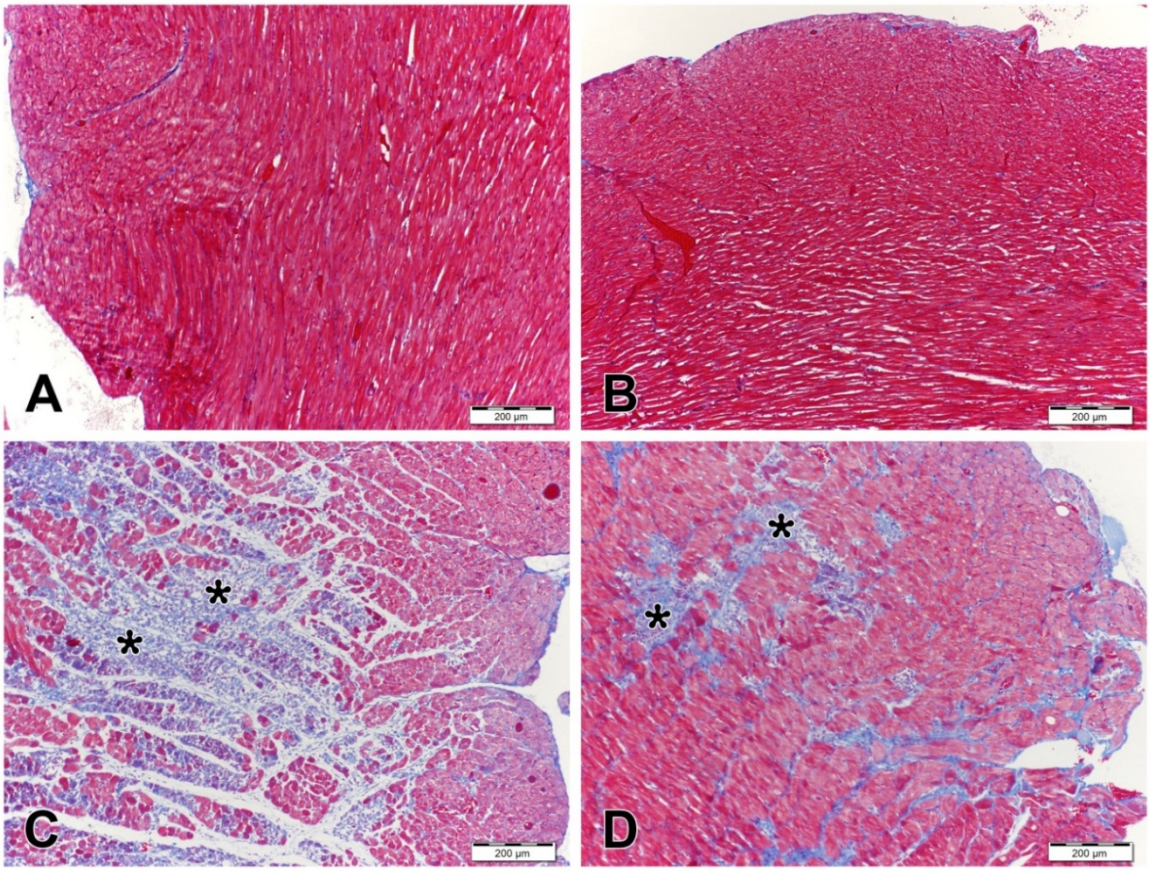


**Fig. 2:** Heart sections stained with Masson's trichrome. (A) Control group, and (B) SIM group showing normal amount and distribution of fibrous tissue (blue color) limited to the endomysium. (C) ISO group showing extensive fibrosis (blue color) in the sub-endocardial areas (asterisks) replacing lost cardiomyoctes. (D) ISO/SIM group showing marked decrease of areas of sub-endocardial fibrosis (asterisks). [Scale bars = 200 µm]

**Table 7:** Mean area percent of fibrous tissue (as detected by Masson's trichrome) in the studied groups

| | Group I (Control) | Group II (SIM) | Group III (ISO) | Group IV (ISO/SIM) |
|---|---|---|---|---|
| Mean ± SE | 2. 180 ± 0.205 | 3.006 ± 0.453 | 22.268 ± 2.015 | 13.031 ± 3.273 |
| *P1* | | 0.957 | 0.002* | 0.112 |
| *P2* | 0.957 | | 0.002* | 0.119 |
| *P3* | 0.002* | 0.002* | | 0.166 |

SE, standard error; *P1*, versus group I; *P2*, versus group II; *P3*, versus group III;
* significant difference ($P \leq 0.05$)

### 3.5. Immunohistochemical analysis

Immunostaining with anti-CD68 antibody showed large areas of strongly immunopositive inflammatory cells in necrotic areas of heart sections from ISO-injected rats (group III) (Fig. 3C) as compared to either control (group I) or SIM (group II) rats (Fig. 3 A and B). SIM treatment (group IV) significantly reduced these immunopositive patches (Fig. 3D, Table 8).

## 4. Discussion

In the present study it was observed that ISO administration leads to deleterious changes in the myocardium. Hypertrophy of cardiomyocytes contributes to left ventricular hypertrophy, which is induced by hypertension (Zheng and Lu, 2015). Therefore, elucidation of the mechanisms underlying the development and regulation of cardiomyocytes hypertrophy is of great importance in the prevention of cardiovascular diseases. Sustained adrenergic stimulation is an important hallmark of the maladaptive cardiac hypertrophy.

The cardiac hypertrophy induced by the β1-adrenergic receptor agonist, ISO, mimics this sustained adrenergic stimulation and it represents the widely used model (Molojavyi et al., 2010). ISO has been reported to induce infarct-like necrosis of the heart muscle (Ennis et al., 2003). It is also known to generate free radicals and to provoke lipid peroxidation leading to irreversible damage to the myocardial cell membrane (Sathish et al., 2003).

In addition, the activation of β-adrenergic signaling induces different mechanisms including enhanced protein synthesis and stimulation of mitogen-activated protein kinases and phosphatidylinositol-3 kinases, which contribute to the hypertrophic phenotype (Zhang et al., 2003).

On the eighth day of ISO administration, in this experiment, rats exhibited myocardial hypertrophy as evidenced by the significant increase in HW and

HW/BW ratio. In this context, Taylor and Tang (1984) reported that maximum HW can be reached on the eighth day of subcutaneous ISO administration. The observed increase of HW in ISO-injected rats might be attributed to the increase in water content, edema of the interstitium, and inflammatory cellular infiltration due to extensive necrosis of cardiac muscle (Patel et al., 2010). The ISO-induced cardiac hypertrophy, in this study, was further confirmed by the increased thickness of the left ventricular wall, mononuclear cellular infiltration, and the excessive fibrous tissue deposition between cardiomyocytes of the left ventricle. Pre-treatment with SIM significantly decreased the HW of ISO-injected rats, which is consistent with the observations of Liu et al. (2008) who reported that SIM prevents cardiac hypertrophy in vitro and in rats with pressure overload due to an abdominal aortic constriction. Takayama et al. (2006) referred the attenuating effect of SIM on cardiac hypertrophy to its anti-inflammatory role.

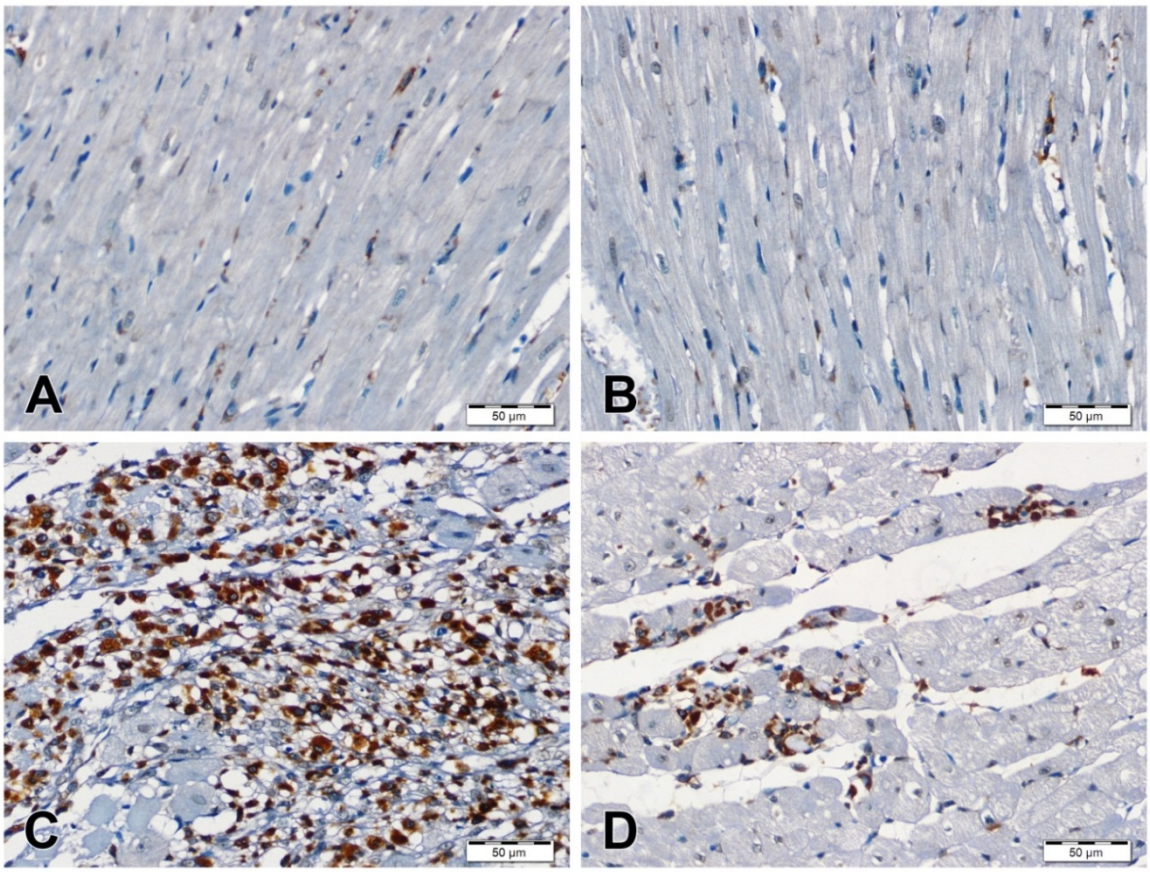


**Fig. 3:** Heart sections immunostained for CD68. (A) Control group, and (B) SIM group showing few immunostained cells (brown color) in the endomysium between myocardial cells. (C) ISO group showing large focal sub-endocardial patches with strongly immunopositive collections of cells. (D) ISO/SIM group showing marked decrease of size of immunopositive cellular patches. [Scale bars 50 µm]

**Table 8:** Mean area percent of CD68 immunopositivity in the studied groups

| | Group I (Control) | Group II (SIM) | Group III (ISO) | Group IV (ISO/SIM) |
|---|---|---|---|---|
| Mean ± SE | 5.098 ± 0.462 | 3.550 ± 0.472 | 48.665 ± 1.355 | 15.767 ± 0.700 |
| *P1* | | 0.167 | 0.000* | 0.000* |
| *P2* | 0.167 | | 0.000* | 0.000* |
| *P3* | 0.000* | 0.000* | | 0.000* |

SE, standard error; *P1*, versus group I; *P2*, versus group II; *P3*, versus group III;
* significant difference ($P \leq 0.05$)

There is considerable controversy about the BP changes due to the ISO-induced cardiac injury. Multiple studies have demonstrated declined pumping ability of the heart (Lin, 1973; Baldwin et al., 1982) and depressed contractile ability of the heart in ISO-induced hypertrophy (Vassallo et al., 1988; Bowling et al., 1990; Stein et al., 1996), while others reported contractile function enhancement (Cihak et al., 1992; Tang and Taylor, 1996; Arthur and Belcastro, 1997). In the present study, rats with hypertrophic hearts exhibited significant elevation in both systolic and diastolic BP, which is in agreement with Papparella et al. (2008), who reported increased BP in angiotensin-II–treated rats. On the other hand, SIM pre-treated rats that suffered from ISO-induced hypertension showed decreased systolic and diastolic BP. ISO has been reported to be associated with increases in serum and myocardial lipids, which in turn lead to coronary heart disease (Prince and Rajadurai, 2005). Therefore, the

ameliorative effect of SIM against ISO-induced cardiac hypertrophy might be partially connected to its hypolipidemic and myocardial-enhancing effects. On the contrary, Adameova et al. (2009) revealed that SIM alleviates myocardial contractile dysfunction in rat heart independent of its cholesterol-lowering effects. Statins are well accepted to not only have satisfactory lipid regulatory effects, but also inhibit the proliferation of vascular smooth muscle cells and cardiac fibroblasts (Wang et al., 2013). Thus, the positive therapeutic effects of statins are not limited to their cholesterol-lowering effects.

Inflammatory response and cytokine elaboration are integral components of the host immune response to tissue injury and play an active role after myocardial infarction (Nian et al., 2004). Cardiac ischemia leads to many intracellular changes involving disruption of mitochondrial membrane potential, leading to the formation of oxygen free radicals or ROS, initiating generation of different oxygen intermediates that directly damage cellular DNA, proteins and lipids and activating pathways of stress response resulting in the production of TNF-α that stimulates secretion of IL-6 that increases production of NF-κB (Arslan et al., 2010). Cardiac markers are the useful tool for detection of myocyte damage and have great clinical importance (Jaffe et al., 1996). Changes in cardiac markers (troponin-T and CPK-MB) and inflammatory markers (IL-6 and TNF-α) have been detected in this experiment. CPK-MB and troponin are the early markers for detection of myocardial ischemic injury (Goyal et al., 2010). Administration of SIM could significantly return the altered levels of both cardiac and inflammatory markers as a result of to its anti-inflammatory effect (Takayama et al., 2006).

Histopathological examination of heart tissue from control (group I) and SIM (group II) groups revealed clear integrity of the myocardial cells and the endomysium without evidence of focal necrosis or inflammatory cellular infiltration. Heart tissue from ISO group (group III) showed sub-endocardial edema, patches of myocardial degeneration and cellular infiltration, in addition to extensive fibrosis. Brooks and Conrad (2009) reported that treatment with ISO resulted in marked myocyte loss and increased fibrosis that were limited to the sub-endocardium of the left ventricle free wall and septum. Hypertension produces collagen deposition, changes referred to as myocardial fibrosis, which leads to depressed myocardial performance (Bu et al., 2008). Some studies indicated that ISO-induced acute myocardial infarction after ischemia is caused by myocardial hyperactivity and coronary hypertension (Yeager and Iams, 1981), the inflammatory response (Steffens et al., 2009) and cell apoptosis (Tanaka et al., 2004). In this study, the reduced inflammatory cellular infiltration, myocardial necrosis, and fibrosis in SIM pre-treated rats (group IV) confirmed the cardio-protective effect of SIM.

After the expression of pro-inflammatory cytokines, the process of myocyte apoptosis, induced by TNF-α (Krown et al., 1996) via binding to TNFR-1 that contains a death domain (Screaton and Xu, 2000) occurred in myocardium (Zhang et al., 2006). Cardiac apoptosis can proceed via either death-receptor or mitochondrial-dependent pathways, either of which activates specific caspases resulting in cell death. The death-receptor pathway proceeds when extracellular cell signal ligands, such as TNF-α, FasL, Apo-3L and TNF-related apoptosis-inducing, bind to their specific cell membrane receptors (Haunstetter and Izumo, 1998).

The present study also revealed increased immune expression of the antibody to monocyte/macrophage antigen CD68 in the heart of ISO-injected rats, which confirms an increase of the macrophages influx. This finding coincides with Chen et al. (2013) who discovered an increase of number of $CD68^+$ cells in spontaneous hypertension rats, and with Chang et al. (2013) who confirmed that monocytes/macrophages dominate the cellular infiltrate for the first two weeks after MI and participate in infarct wound healing.

Watts et al. (2008) demonstrated the role of inflammation in right ventricular damage. They found that heart sections showed clusters of $CD68^+$ cells one day after pulmonary embolism. Examination of heart sections one week after pulmonary embolism showed that $CD68^+$ cells are prevalent throughout the region of right ventricle outflow tract. These cells were found throughout the loose connective tissue region of the outflow tract by week 3 after pulmonary embolism. The $CD68^+$ cells appeared to be excluded where fibrous material was observed at week after pulmonary embolism.

In ischemia-reperfusion injury, any inflammation is likely harmful. Neutrophils and monocytes/macrophages release proteolytic enzymes and reactive oxygen species and exacerbate the injury by harming myocytes that survived the ischemic period. Preclinical studies have shown that anti-inflammatory treatment can be beneficial because it decreases the infarct size-to-area-at-risk ratio after ischemia-reperfusion injury (Steffens et al., 2009).

In conclusion, the findings of this study demonstrate that SIM prevents ISO-induced cardiac hypertrophy and infarction in rats through attenuating influx of inflammatory cells as monocytes/macrophages. Thus, SIM might be considered a potential candidate as an anti-inflammatory drug in myocardial ischemia and infarction.

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