Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.234154.2873 Volume 30, Issue 8, Nov. 2024
Manuscript ID ZUMJ-2401-3135 DOI . 10.21608/ZUMJ.2024.265340.3135

ORIGINAL ARTICLE

The Possible Cardio protective Effect of Ivabradine versus Bisoprolol on Myocardial Ischemia/Reperfusion Injury in Rats

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Submit Date 25-01-2024
Revise Date 16-02-2024
Accept Date 16-02-2024

ABSTRACT

Background: Cardiovascular disease is primary cause of death globally, which is primarily caused by coronary artery disease. High resting heart rate is linked to increased cardiovascular and overall death rate, as well as in coronary artery disease and chronic heart failure patients. Decreasing heart rate became a key in the treatment of patients with cardiovascular disease. Our research aimed to evaluate the prophylactic effects of ivabradine on myocardial ischemia reperfusion injury in rats in comparison with bisoprolol. **Methods:** The study was done on 40 adult male albino rats (10 rats in each group) that were divided into Sham-operated group, control diseased Group, ivabradine-pretreated group and bisoprolol-pretreated group. Blood pressure, heart rate, T wave voltage, biochemical tests and infarct size were measured. Representative cardiac samples of each group were also used for histopathological examination using H&E stain as well as immunohistochemical stain with Bax. **Results:** Bisoprolol pretreatment reduced the serum level of creatine kinase isoenzyme MB (CK-MB) compared to the control diseased group. Meanwhile, pretreatment with ivabradine produced more decrease in the serum level CK-MB compared to the control diseased group. Oral pretreatment with ivabradine decreased the percentage of the infarct size as compared to control diseased group. In the bisoprolol-pretreated group, the infarct size of the left ventricle was lower than that of control diseased but higher than the ivabradine-pretreated group. **Conclusions:** It can be concluded from the previous findings that ivabradine possesses protective effect against I/R injury in rats, as evidenced by significant decrease of each of the % of infarct size, T-wave voltage, CK-MB. Thus, ivabradine can be used if bisoprolol is contraindicated.

Keywords: Ivabradine; Bisoprolol; Myocardial Ischemia; Reperfusion Injury.

INTRODUCTION

Ischemic heart disease is one of the greatest health threats to people and considered as a major cause of death worldwide**.** Myocardial reperfusion is the main treatment for myocardial ischemia**.** Despite the widespread use of percutaneous coronary intervention in clinical settings, myocardial ischemia/reperfusion injury (I/R) remains poorly understood, which inexplicably leads to severe adverse outcomes such as ventricular fibrillation, acute heart failure and death**.** Therefore, the research for preventative measures for I/R injury is mandatory **[1].** One important factor affecting the supply and demand of oxygen in the heart is heart rate (HR). A high resting heart rate is associated with an increased risk of cardiovascular disease and overall mortality in the general population, as well as in patients with coronary artery disease and chronic heart failure. Therefore, there is a need for novel, safe, and efficient medications to regulate heart rate **[2].**

At this moment, the drugs most used to clinically lower heart rate are beta-blockers. However, using beta-blockers while suffering from peripheral vascular disease, asthma, atrioventricular block, decompensated congestive heart failure, or hypotension might be dangerous. Beta-blockers have several negative effects and contraindications in addition to their negative chronotropic effect, negative inotropic action, and negative dromotropic activity. These drawbacks have led to the creation of pure HR-reducing substances like ivabradine **[3].**

Ivabradine is a selective inhibitor of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which lowers heart rate (HR). By inhibiting the heart's I_f-current, ivabradine regulates the heart rate by preventing the sinus node's spontaneous diastolic depolarization. Ivabradine has no effect on intra-atrial, atrioventricular, or intraventricular stimulus conduction; instead, its cardiac effects are exclusive to sinus nodes. It doesn't change ventricular repolarization or myocardial contractility, and it doesn't significantly affect blood pressure **[4].**

It has been demonstrated that ivabradine dramatically lowers the oxygen consumption of the heart, enhances left ventricular systolic function, and reduces inflammatory markers, left ventricular hypertrophy, and fibrosis. These effects all help to lower the morbidity and mortality associated with cardiovascular diseases. Currently, the medication is licensed to treat chronic heart failure and chronic stable angina pectoris in individuals with coronary artery disease who have normal sinus rhythm and heart rate of at least 70 beats per minute **[5].**

METHODS

- **Experimental animals**

Forty adult male albino rats, weighing between 250 and 300 grams, were used for the investigation. They were acquired from the animal house of Faculty of Veterinary Medicine- Zagazig University. On December 29, 2022, Zagazig University's Institutional Animal Care and Use Committee (IACUC) ZU-IACUC/3/F/410/2022 granted ethical

permission for this work.The rats were accommodated to animal house conditions for one week before the experiments going on. They were housed under standard environmental conditions (temperature, 22±2^{0}C; humidity, 50%-55%; 12-hour light/ 12-hour dark cycle) in plastic cages with wood shave bedding, each cage containing 10 rats. We selected only adult male albino rats weighing 250-300 gm per each and we excluded female rats.

- **Experimental design**

After acclimation for 1 week 40 rats were divided randomly into 4 groups: **Sham operated group**: 10 rats received distilled water, 1ml/100gm rat daily, orally by gavage for 6 days then surgery was performed without coronary artery ligation. **Control diseased group**: 10 rats received distilled water, 1ml/100gm rat daily, orally by gavage for 6 days then myocardial ischemia reperfusion injury was induced by coronary artery ligation then reperfusion followed. **Ivabradine-pretreated group:** 10 rats received ivabradine (Sigma-Aldrich, USA) in a dose of 9.4mg/kg/day orally by gavage for 6 days. The utilized dose is equimolar to bisoprolol 8.8mg/kg/day and approximate to dose of 10mg/kg/day**[6].Bisoprolol-pretreated group:** 10 rats receive bisoprolol (Sigma-Aldrich, USA) in a dose of 8.8mg/kg/day orally by gavage for 6 days. The utilized dose is equimolar to the ivabradine dose 9.4mg/kg/day and approximate to the 10mg/kg/day **[7].** The coronary ligation was performed 2 hours after the last dose on the 6th day of the study **[3].**

- **Myocardial ischemia reperfusion model:**

Urethane (Prolabo, Paris, France) was injected intraperitoneally into the rats at a dose of 2 grams/kg to induce anesthesia. The chest was opened by a middle thoracotomy, followed by a pericardiotomy. To ligate the left anterior descending artery, the heart was exposed via a middle thoracotomy at the 4th intercostal space. A 6–0 Prolene loop along with a snare occlude were passed around the left coronary artery. Successful coronary occlusion was indicated when a visible blanched area that was distal to the ligation site was observed **[8].**

The rats in the control diseased group experienced ischemia for 30 minutes, followed by 2 hours of reperfusion. The same surgical technique as previously described was performed on sham-operated rats, except for coronary artery ligation **[9].** Before coronary artery ligation, immediate after ligation, and every 30 minutes during reperfusion up to the experiment's end, blood pressure was recorded.

- **Hemodynamic parameters**

The carotid artery was cannulated to measure arterial blood pressure with a pressure transducer. ECG was monitored via subcutaneous stainless-steel needle electrodes. ECG parameters measured include heart rate (beats/m) and T-wave voltage (mV). MABP and ECG were recorded before coronary artery ligation, immediately after ligation and every 30 minutes during reperfusion up to the experiment's end. We used Power Lab (4/35) data acquisition system (ADInstruments, Castle Hill, Australia, Pty Ltd.) for MABP and ECG monitoring. Data were extracted and analyzed

by LabChart7 software before coronary ligation and 2 hours after reperfusion.

- **Sample collection**

Following every experiment, blood samples were taken for biochemical research. Using a rat arterial polyethylene cannula, arterial blood samples were obtained and placed in sterile, dry test containers. The samples were centrifuged for 15 minutes at 3000 rpm. Aliquots of serum were taken and kept at around -20°Cfor subsequent biochemical analysis of various parameters, such as serum CK-MB, Tumor necrosis factor (TNF-α), Malondialdehyde (MDA), superoxide dismutase (SOD) and reduced glutathione (GSH).

Hearts were excised for assessment of infarct size (in five rats in each group). Heart slides of other five rats in each group were stained by Haematoxlyin and eosin (H&E) stain as well as immunohistochemical staining using BAX and examined with light microscope under different magnification powers.

- **Biochemical measures**

Serum CK-MB (Bioassay technology laboratory, Spinreact, Spain, Catalogue no: CSC E-17176), TNFα (CUSABIO, USA. Catalogue no: CSB-E11987r), MDA (MYBIOSOURCE, USA. Catalogue no: MBS268427), SOD (CUSABIO, USA. Catalog no: CSB-E08555r) and GSH (CUSABIO, USA. Catalog no: CSB-E12144r) were assessed using the enzyme linked immunosorbent (ELISA) kits

- **Measurement of infarction size:**

The beating hearts were removed at the end of each experiment and placed in normal saline solution. After the heart was finely dissected to release it from surrounding tissues and large veins, each heart of the coronary ligated rats was cut into three transverse slices, each measuring approximately 1.5 mm in thickness, starting at the level of the ligation and ending at the apex. The sections were cut with a sharp surgical razor. After that, the slices were dyed for 15-20 minutes at 37^0 C using a 1.5% TTC (2,3,5-Triphenyltetrazolium chloride, Merck KGaA, Darmstadt, Germany) in phosphate buffer pH 7.4. When dehydrogenase enzyme systems are intact, red precipitates are formed by the TTC stain. Thus, the non-infarcted (normal) tissue-stained crimson red. While areas of necrosis that lack dehydrogenase activity failed to stain and remained pale (white or light yellow in color). Thus, the area of infarction is clearly discernible and thus quantifiable **[3].**

Once the color has been established, fix the slices in 10% formalin for 20 minutes to increase the contrast. Then clear glass plates were placed over both sides of each slice. We then photograph the tissue using digital camera for a permanent record. A Media Cybernetics, Wyoming, USA device called the Image-Pro Plus 7.0 was used to measure the areas that were stained red and white. The following equation was used to get the proportion of infarction size: size of infarct % = size of infarct / total size of slice x 100 **[10].**

- **Histopathological and immunohistochemical studies**

Heart slides of other five rats in each group were stained by Haematoxlyin and eosin (H&E) stain according to manufacturer's protocols. The staining results were observed under light microscope at 400× magnifications. As well as

immunohistochemical staining using BAX examined with light microscope at 400× magnifications

STATISTICAL ANALYSIS

Means±standard error (SE) was tabulated for the acquired results. One-way analysis of variances (one-way ANOVA) was used to compare data between groups, and the Post-Hoc Tukey test was used after, as explained by **[11].** When $p < 0.05$, the differences were deemed significant. The statistical analysis was performed using the statistical package of social sciences (SPSS) software (version 26).

RESULTS

- **The mean arterial blood pressure (MABP) in sham-operated, controldiseased, ivabradine-pretreated and bisoprolol-pretreated groups before, immediately after coronary artery ligation and at 120 minutes after reperfusion (Table. 1)**:

Before the operation:

The MABP in the sham operated, control diseased and ivabradine-pretreated groups were insignificantly different in relation to each other while in the bisoprolol-pretreated group, the MABP was significantly decreased in relation to all groups.

Immediately after operation:

The MABP in the sham operated, control diseased and ivabradine-pretreated groups were insignificantly different in relation to each other while in the bisoprolol-pretreated group, the MABP was significantly decreased in relation to all groups.

120 minutes after reperfusion:

The MABP in the sham operated, control diseased and ivabradine-pretreated groups were insignificantly different in relation to each other. In the bisoprolol-pretreated group, the MABP was significantly decreased in relation to all groups.

- **HR changes (beat/min) in sham-operated, control diseased, ivabradine-pretreated and bisoprolol-pretreated groups before, immediately after coronary artery ligation and at 120 minutes after reperfusion: (Table 2)**

Before the operation:

The baseline HR in the sham operated and control diseased groups were insignificantly different in relation to each other. In the ivabradine-pretreated group, the baseline HR was significantly decreased in relation to all other groups. In bisoprolol-pretreated group, the baseline HR was significantly decreased in relation to sham operated and control groups.

Immediately after ligation ofcoronary artery:

The HR in the sham operated group was significantly different in relation to other groups. In the control diseased group, the HR was significantly increased in relation to all other groups. In ivabradine-pretreated group, the HR was significantly decreased in relation to all other groups, while in the bisoprolol-pretreated group the HR was significantly decreased in relation to sham and control diseased groups, while was significantly increased in relation to ivabradine-pretreated group.

120 minutes after reperfusion:

The HR in the sham operated group was significantly higher than control diseased group and significantly lower than ivabradine-pretreated and bisoprolol-pretreated groups. In the control diseased group, the HR was significantly increased in relation to all other

groups. In ivabradine -pretreated group, the HR was significantly decreased in relation to all other groups. In the bisoprolol-pretreated group the HR was significantly decreased in relation to all other groups while was significantly increased in relation to ivabradine-pretreated group.

- **T wave voltage (mV) changes in sham-operated, control diseased, ivabradine-pretreated and bisoprolol-pretreated groups before, immediately after coronary artery ligation and at 120 minutes after reperfusion: (Table 3):**

Before the operation:

The baseline T wave voltage in the sham operated, control diseased, ivabradine-pretreated and bisoprolol-pretreated groups were insignificantly different in relation to each other.

Immediately after ligation ofcoronary artery:

The T wave voltage in the sham operated group was significantly decreased in relation to other groups while in the control diseased group, the T wave voltage was significantly increased in relation to all other groups. In ivabradine-pretreated group, the T wave voltage was significantly increased in relation to sham operated. While in the bisoprolol -pretreated group, the T wave voltage was significantly increased in relation to sham operated, but the T wave voltage in ivabradine and bisoprolol groups were insignificantly different in relation to each other.

120 minutes after reperfusion:

The T wave voltage in the sham operated group was significantly decreased in relation to other groups while in the control diseased group, the T wave voltage was significantly increased in relation to all other groups. In ivabradine-pretreated group, the T wave voltage was significantly increased in relation to sham operated group. While in the bisoprolol-pretreated group, the T wave voltage was significantly increased in relation to sham operated group, but the T wave voltage in ivabradine-pretreated and bisoprolol-pretreated groups were insignificantly different in relation to each other.

- **Effects of different treatments on the serum creatine kinase isoenzyme MB (CK-MB) level:(Table 4).**

Serum CK-MB level in the control diseased group was significantlyhigher than that in the sham operated group. Also, it was significantly higher than ivabradine-pretreated and bisoprolol-pretreated groups. The serum level of CK-MB in the ivabradine-pretreated group was significantly lower than that of the control diseased group and that of the bisoprolol-pretreated group. However, this value is still significantlyhigher than that of the sham-operated group**.** The bisoprolol-pretreated group's serum level of CK-MB was significantly lower than that of the disease-control group. Serum CK-MB level, however, remained significantly greater than those of the ivabradine and sham-operated groups.

- **Effects of different treatments on the serum (TNF-α) level:(Table 4).**

Serum TNF-α level in the control diseased group was significantly higher than that in the sham operated group. It was also significantly higher than that of the ivabradine-pretreated and bisoprolol-pretreated groups. The ivabradine-pretreated group's serum TNF-α

level was significantly lower than its level in control diseased group. This value is still significantly higher than sham-operated group. In the bisoprolol-pretreated group, serum TNF-α level was significantly lower than its level in control diseased group. Serum TNF-α level was significantlyhigher than that of the sham operated group. Ivabradine and bisoprolol-pretreated groups were insignificantly different in relation to each other.

- **Effects of different treatments on the serum (MDA) level:(Table 4).**

  The MDA serum level in control diseased group was significantly higher than that in the sham operated group. It was also significantly higher than that of the ivabradine-pretreated and bisoprolol-pretreated groups. In the ivabradine-pretreated group, serum MDA level was significantly lower than that of bisoprolol pretreated and the control diseased groups. This value is still significantly higherthan the sham-operated group. In the bisoprolol pretreated group, serum MDA level was significantlylower than control diseased group. Serum MDA level, however, remained significantly higher than those of the ivabradine -pretreated and sham-operated groups.

- **Effects of different treatments on the serum (SOD) level:(Table 4).**

  Serum SOD level in the control diseased group was significantly lower than that of all other groups.In the ivabradine-pretreated group, serum level of SOD was significantlyhigher than bisoprolol group and control diseased group. This value is still significantly ($p<0.05$) less than the sham group.In the bisoprolol-pretreated group, serum level of SOD was significantly higher than the control diseased group. Serum SOD level was significantly lower than those of the ivabradine-pretreated and sham-operated groups.

- **Effects of different treatments on the serum (GSH) level:(Table 4).**

  Serum GSH levels in the control diseased group was significantly lower than those in all other groups.In the ivabradine-pretreated group, serum level of GSH was significantly higher than bisoprolol-pretreated and control diseased groups. This value is still significantly less than the sham-operated group.The serum level of GSH in the bisoprolol-pretreated group was significantly higher than that of control diseased group. However, serum GSH level was still significantly lower than that of the sham-operated group and ivabradine-pretreated group.

- **Effects on infarct size of left ventriclein sham-operated, controldiseased, ivabradine-pretreated and bisoprolol-pretreated groups:(photo1).**

  The infarct size in the control diseased group was 51.4±0.51 % of the total surface area of the left ventricle, while in ivabradine-pretreated group was 32.8±0.58 %, which was significantly ($p<0.05$) lower than that of the control diseased group. In the bisoprolol-pretreated group, the infarct size of the left ventricle was 37.8±0.58 % of the total surface area of the left ventricle, which was significantly ($p<0.05$) lower than that of control diseased but significantly higher than ivabradine-pretreated group.

- **Histopathological and immunohistochemical results**

Hematoxylin and Eosin Photomicrographs for microscopic changes in the cardiac tissue in sham-operated, control, ivabradine and bisoprolol groups: (photo2)

In Sham operated group cardiac tissue showed normal appearing in cardiac muscle with longitudinal cut section and intact blood vessels, in control diseased group cardiac tissue showed foci of necrotic muscle and dilated congested blood vessels. In ivabradine- pretreated group cardiac tissue showed normal cardiac muscle with transverse cut section indicating improvement of muscle necrosis and marked improvement in vascular congestion, while in bisoprolol pretreated group cardiac tissue showed focal mild cardiac muscle necrosis with some foci showing pycnotic nuclei indicating mild improvement of muscle necrosis and improvement of vascular congestion but not the dilatation.

Photomicrographs of immunohisto-chemical staining, Expression of Bax protein for microscopic changes in cardiac tissue in sham-operated, controldiseased, ivabradine-pretreated and bisoprolol-pretreated groups:(Photo 3).

In Sham operated group cardiac tissue showed negative BAX immunohistochemical stain indicating no cardiac muscle apoptosis, while in control diseased group cardiac tissue showed strong and marked BAX immunohistochemical stain indicating marked cardiac muscle apoptosis. In ivabradine - pretreated group cardiac tissue showed negative BAX immunohistochemical stain indicating good improvement of cardiac muscle apoptosis, while in bisoprolol-pretreated group the tissue showed weak cytoplasmic BAX immunohistochemical stain indicating mild improvement of cardiac muscle apoptosis.

Table (1): The mean arterial blood pressure (mmHg) in sham operated, control diseased, ivabradine-pretreated and bisoprolol-pretreated groups before, immediately after coronary artery ligation and at 120 minutes after reperfusion**:**

	Sham operated group N=10	**Control diseased group N=10**	**Ivabradine-pretreated group N=10**	**Bisoprolol-pretreated group N=10**
Before operation	95.4±2.01^{A}	94.8±2.14^{A}	93.9±1.42^{A}	85.5±2.01^{B}
Immediate after ligation	88.6±1.66^{A}	85.1±1.84^{A}	88.2±1.08^{A}	79.6±1.84^{B}
120 min. after reperfusion	85.8±1.76^{A}	81.1±1.77^{A}	85.1±1.08^{A}	75.9±1.75^{B}

- *Values represent means ± standard error of mean arterial blood pressure.*
- *In the same row, values without common superscript capital letters are significantly different ($p<0.05$)*
- *N is number of rats in each group*

Table (2): Heart rate changes (beats/min) in sham operated, control diseased, ivabradine-pretreated and bisoprolol-pretreated groups before, immediately after coronary artery ligation and at 120 minutes after reperfusion**:**

	Sham operated group N=10	**Control diseased group N=10**	**Ivabradine-pretreated group N=10**	**Bisoprolol-pretreated group N=10**
Before operation	305±3.99^{A}	313±3.65 ^{A}	233±1.70 ^{B}	273±2.38 ^{C}
Immediate after ligation	357±4.3 ^{A}	374±3.42 ^{B}	263±5.64 ^{C}	311±2.08^{D}
120 min. after reperfusion	316±3.99 ^{A}	363 ±2.79 ^{B}	254±2.00^{C}	294±9.50 ^{D}

- *Values represent means ±standard error of Heart rate.*
- *In the same row, values without common superscript capital letters are significantly different ($p<0.05$)*
- *N is number of rats in each group*

Table (3): The changes of the T wave voltage (mV) in sham operated, control diseased, ivabradine-pretreated and bisoprolol-pretreated groups before, immediately after coronary artery ligation and at 120 minutes after reperfusion**:**

	Sham operatedgroup N=10	**Controldiseased group N=10**	**Ivabradine-pretreated group N=10**	**Bisoprolol-pretreated group N=10**
Before operation	0.0579±0.00 ^{A}	0.057±0.006^{A}	0.053±0.006^{A}	0.057±0.005^{A}
Immediate after ligation	0.0569±0.003 ^{A}	0.223±0.018 ^{B}	0.130±0.005 ^{C}	0.154±0.007 ^{C}
120 min. after reperfusion	0.0550±0.004 ^{A}	0.189±0.02^{B}	0.11±0.007^{C}	0.127±0.006^{C}

- *Values represent means ±standard error of T wave voltage.*
- *In the same row, values without common superscript capital letters are significantly different ($p<0.05$)*
- *N is number of rats in each group*

Table (4): The serum levels of CK-MB, TNF-α, MDA, SOD and GSH in sham operated, control diseased, ivabradine-pretreated and bisoprolol-pretreated groups**:**

	Sham operated group N=10	**Controldiseased group N=10**	**Ivabradine-pretreated group N=10**	**Bisoprolol-pretreated group N=10**
CKMB (U/L)	164.4±11.5 ^{A}	1438.4±130 ^{B}	323.2±12.2 ^{C}	523±16.9 ^{D}
TNF-α (Pg/ml)	294.3±16.4 ^{A}	1075.4±71.5 ^{B}	508.3±10.4 ^{C}	594.9±14.7 ^{C}
MDA (nmol/ml)	0.92±0.07 ^{A}	14.23±1.3 ^{B}	3.56±0.26 ^{C}	5.92±0.28 ^{D}
SOD (U/ml)	154.9±3.43 ^{A}	26.6±3.48 ^{B}	96.2±2.72 ^{C}	75.4±2.80 ^{D}
GSH (ng/ml)	173.9±5.21 ^{A}	28.2±2.68 ^{B}	126.9±1.56 ^{C}	96.8±2.59 ^{D}

- *Values represent means ±standard error of the serum levels of CK-MB, TNF-α, MDA, SOD and GSH*
- *In the same row, values without common superscript capital letters are significantly different ($p<0.05$)*
- *N is number of rats in each group*

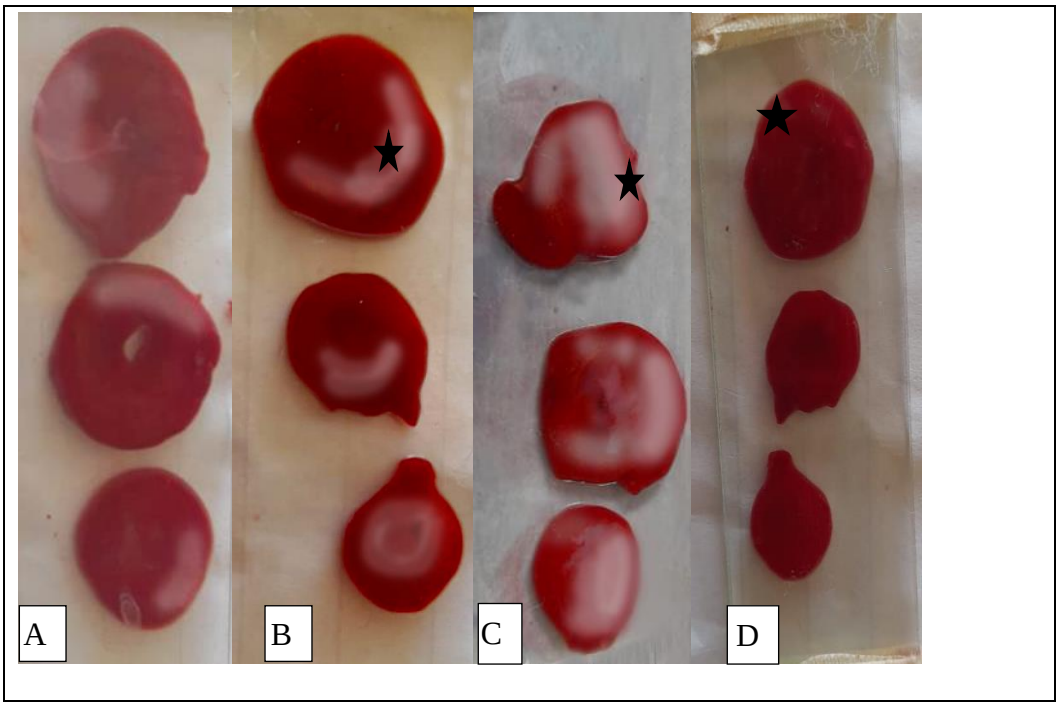

Photo (1): Images of TTC stained cross-section of the rats' hearts showing effects of different treatments on myocardial infarct size after ischemia reperfusion injury.

- Star: refer to the infarcted myocardium.
- A: Shamoperated group B: Control diseased group
- C: Ivabradine-pretreated group D: Bisoprolol-pretreated group

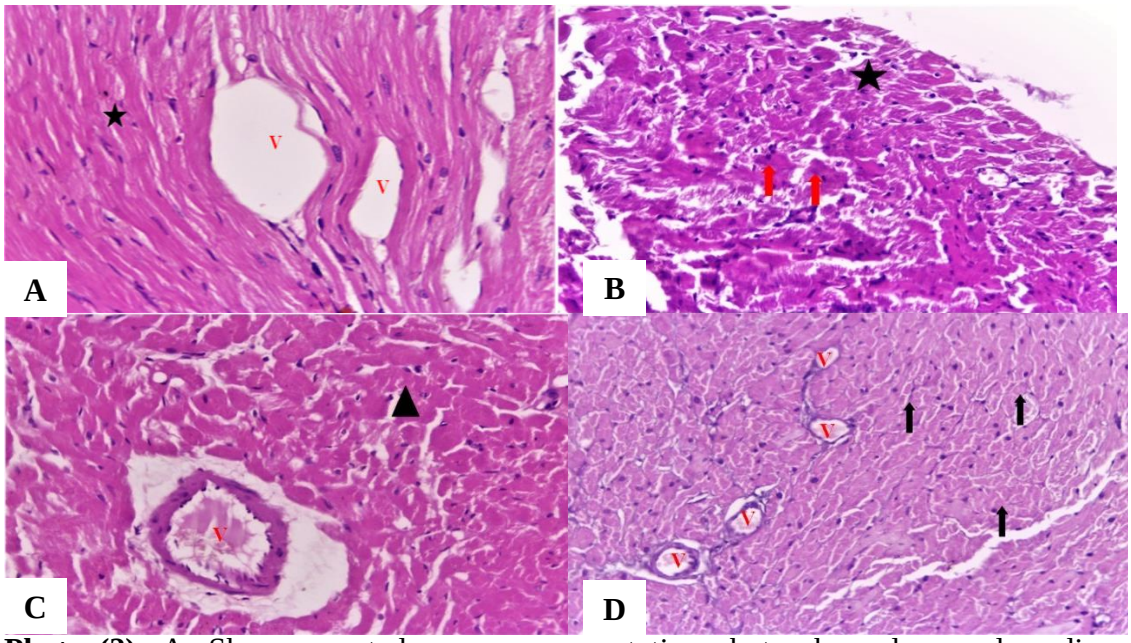

Photo (2): A: Sham operated group, representative photo showed normal cardiac muscle with longitudinal cut section (star) and intact blood vessels (V). B: Control diseased group, representative photo showed cardiac muscle necrosis (star) with pycnotic nuclei (red arrows) and deep cytoplasmic eosinophilia. C: Ivabradine-pretreated group, representative photo showed normal cardiac muscle with transverse cut section (arrowhead) indicating improvement of muscle necrosis and marked improvement in vascular congestion (V). D: Bisoprolol pre-treated group, representative photo showed mild improvement in cardiac muscle necrosis with some foci showing pycnotic nuclei (black arrows) and mild necrosis and mild vascular congestion (V) (H&Ex400).

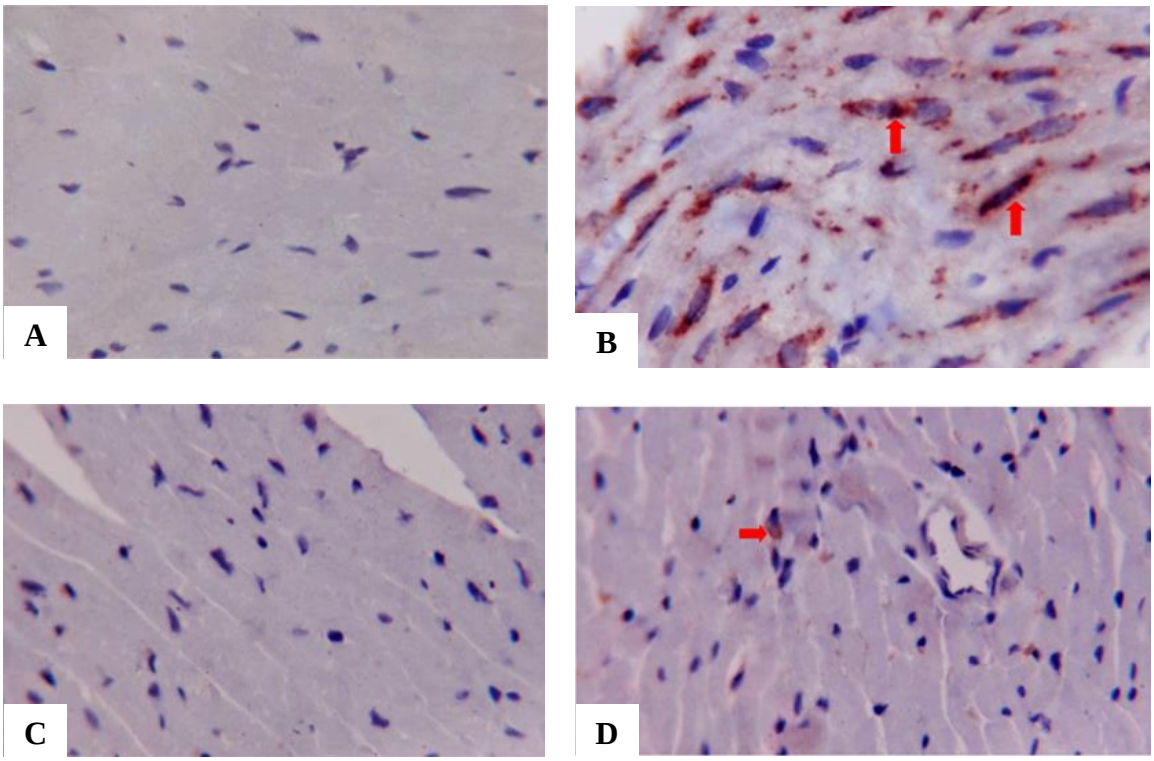

Photo (3): A: Sham operated group, representative photo showed negative BAX immunohistochemical stain indicating with no cardiac muscle apoptosis. B: Control diseased group, representative photo showed strong and marked cytoplasmic BAX immunohistochemical stain (red arrows) indicating marked cardiac muscle apoptosis. C: Ivabradine -pretreated group, representative photo showed negative BAX immunohistochemical stain indicating good improvement of cardiac muscle apoptosis. D: Bisoprolol pre-treated group, representative photo showed weak cytoplasmic BAX immunohistochemical stains (red arrow) indicating mild improvement (IHC BAX stain x 400).

DISCUSSION

Globally, acute myocardial infarction is the most frequent cause of chronic heart failure and one of the main causes of death **[13].** Treatment options for cardiovascular events include coronary bypass surgery, percutaneous transluminal coronary angioplasty, and thrombolysis. Nevertheless, I/R injury could happen when coronary blood flow is restored **[14].** Consequently, it is essential to do research on I/R injury prevention techniques **[1].**

The risk of death is reduced, and the ischemic myocardium's blood supply is restored with early treatment of acute myocardial infarction. Myocardial I/R injury is the term used to describe the more severe damage that is done to the original ischemic myocardium when the interrupted myocardial blood supply is restored within a specific time frame. Oxidative stress is one of the pathophysiological pathways causing I/R injury **[15].** The latter was assessed in the present work by the reduced serum levels of SOD, GSH and the elevated serum level of MDA. **Wang et al.[16]** observed that inflammation is involved in the process of cardiovascular remodeling after MI which was determined in the present study by the elevated serum level of TNF-α.

A significant amount of cardiomyocyte necrosis and apoptosis is one factor contributing to the poor prognosis of patients with acute myocardial infarction **[17],** which was determined in the present work by the immune-histo-chemistry expression of Bax protein.

In the present study, the coronary artery ligation reduced MABP. This decrease may be explained by development of cardiogenic shock or low cardiac output state **[18].** In agreement with these results, **Mahmoud et al. [3]** reported that MABP was decreased in control MI group when recorded after 4 hours following coronary artery ligation compared to the baseline value**.** In addition, **Kamel [19]** found that the coronary artery ligation reduced MABP.

Pretreatment with ivabradine did not produce any changes in the MABP measurements before or after I/R compared to values occurring in the control diseased and sham operated groups. These findings were in accordance with **Joannides et al. [20]** who demonstrated that despite the marked HR reduction, ivabradine does not modify blood pressure. This could be attributed to decrement in the HR without affecting myocardial contractility or vascular tone **[21]**

The present work demonstrated decrement in the baseline MABP in the bisoprolol-pretreated rats as compared to the baseline values of sham-operated, control diseased and ivabradine-pretreatedgroups. These findings are in line with that obtained by **Suojanen et al. [22]** who reported that MABP decreased after bisoprolol treatment when compared with placebo during studying hemodynamic influences of bisoprolol in hypertensive middle-aged men. Bisoprolol is a selective β1 blocker drug. It has negative inotropic and chronotropic effects and decreases heart contraction and heart rate **[23].**

In addition, the MABP was decreased in bisoprolol group immediately after coronary artery ligation as well as after reperfusion. These results were in consistence with **Jun et al. [24]**, who demonstrated the effect of bisoprolol in secondary prevention of acute myocardial infarction in patients undergoing percutaneous coronary intervention and found that systolic and diastolic blood pressure were lower in the bisoprolol group. However, the present results did not cope with **Wang et al. [16]** who reported that administration of

bisoprolol (5mg/kg) for 1 week by oral gavage before being exposed to 0.5 h ischemia/4 h reperfusion did not decrease the blood pressure. This discrepancy may be due to using lower dose of bisoprolol (5mg/kg) in comparison with that (8.8mg/kg) utilized in present study.

As regard the HR, the coronary artery ligation increased HR in relation to the sham operated group and then reperfusion significantly decreased it gradually again towards the normal levels. The increase of HR after coronary artery ligation could be attributed to the increased adrenergic activity which is reflected by the elevated plasma concentration of catecholamines **[25].**

The current study demonstrated that the baseline HR of the ivabradine-pretreated group was lower than baseline findings among control diseased group and sham operated group. The HR increased immediately after ligation and decreased again gradually. HR findings were significantly decreased compared to the HR findings of sham operated group at comparable times. This is consistent with the findings of previous experimental study which revealed that HR was lower in the ivabradine-treated mice than in the Vehicle-treated mice on day 5 after MI **[26].**

In addition, **Calcagno et al. [27]** had shown that the HR was decreased after receiving ivabradine 5 mg twice daily for 30 days of therapy, when studying effects of ivabradine on residual myocardial ischemia after percutaneous coronary intervention.

Ivabradine is a new and unique HCN4 inhibitor that is now utilized clinically for chronic stable angina and chronic heart failure. The drop in HR following ivabradine medication may be related to this. It has been discovered that ivabradine lowers the diastolic depolarization of the pacemaker action potential, which lowers heart rate without changing systemic vascular resistance or cardiac inotropy **[28]**. HR reduction has an established role in cardio protection against I/R injury **[26].** Moreover, **Berdeaux [29]** stated that ivabradine can decrease HR in patients in whom β-blockers are not effective**.**

As regard the pretreatment with bisoprolol, the baseline HR was significantly lower than baseline findings among control diseased group and sham operated group. Then the HR significantly increased immediately post-operative and decreased again gradually. HR findings were significantly decreased compared to the HR findings of sham operated group at comparable times in comparison to control diseased group, while was significantly increased in relation to ivabradine-pretreated group. In line of the present study, **Wang [30],** investigated the safety and effectiveness of bisoprolol in the management of cardiac insufficiency and myocardial infarction when patients with MI received bisoprolol 6.25 mg twice a day continuously for 6 months, the heart rate was significantly decreased compared with the control group. In addition, **Hossain [31]** reported that bisoprolol in patients with systolic heart failure and left ventricular systolic dysfunction decreased heart rate with the up titration of bisoprolol 5 mg od/p. o to 10 mg od/p. o after 6months of therapy.

In the control diseased group T wave voltage findings were increased immediately postoperative in comparison to sham group. These results were consistent with that obtained by **Wang et al. [32]** who reported that, shortly after induction of MI by left coronary artery ligation, the ST height and the T-wave voltage of lead II in ECG were significantly increased. The T-wave

amplitude increased within the first 30 minutes following coronary artery occlusion. Also, **Mahmoud et al. [3]** reported that ST height and the T-wave voltage significantly increased in all groups following coronary artery ligation when compared to the baseline values in each group, and these elevations were sustained till the end of the experiments.

Oral pretreatment with ivabradine or bisoprolol produced decrease in the elevated T-wave voltage after the coronary artery ligation as compared to control diseased group. T wave voltage in ivabradine-pretreated and bisoprolol-pretreated groups were insignificantly different in relation to each other, these findings indicate that both drugs have anti-ischemic effects.

Indeed, **Mahmoud et al. [3]** showed that ivabradine taken orally as a pretreatment had a cardioprotective effect against acute MI, as seen by a significant reduction in blood levels of CK-MB, T-wave voltage, and ST height. Thus, according to the findings of the present work and the other reports, improvement of hemodynamics by ivabradine and bisoprolol, could be suggested as mechanisms of the cardioprotective effects of these drugs.

The current study's findings showed that coronary artery ligation caused myocardial necrosis in rats, which was shown by a significant rise in creatine kinase MB isoenzyme levels in the blood. These results align with the results obtained by **Ma et al. [33]** who demonstrated the increased serum levels of lactate dehydrogenase, CK-MB, and cTnI in the rat model of persistent myocardial ischemia induced by ligation of the left anterior descending coronary artery.

In the present research, bisoprolol pretreatment reduced the serum level CK-MB compared to the control diseased group. Meanwhile, pretreatment with ivabradine produced more decrease in the serum level CK-MB compared to the control diseased group. These results coped with those of **Hendawy et al. [34]** who denoted that treatment with ivabradine, or metoprolol produced decrease in serum CK-MB compared to the control group.

The present study's findings demonstrated that the proinflammatory cytokine TNF-α significantly increased plasma levels in response to ischemia-reperfusion injury. **Wang et al. [16]** demonstrated that, in a rat model of MI, the plasma levels of inflammatory factors, such as IL-6 and TNF-α, were markedly higher than those of the control normal group.

In the present results, the increased TNF-α induced by I/R injury coincided with the findings obtained by **Frangogiannis et al. [35]** who discovered that ischemia tissue reperfusion caused an initial inflammatory response that led to necrosis and irreversible damage to vascular endothelial cells. Free radical production and complement activation are brought on by myocardial necrosis, which sets off a cytokine cascade that is started by the release of TNF-α. This inflammatory response is required for healing and scar formation; on the other hand, it exacerbates myocardial injury following ischemia.

Oral pretreatment with ivabradine decrease the serum level of TNF-α, which was lower than that of the control diseased group. These results were in line with **Al-Kuraishy et al. [36]** who showed that TNF-α serum level was reduced in both doses of ivabradine (5 mg/kg and 10 mg/kg) compared to doxorubicin-induced cardiotoxic group in Albino male mice.

Oral pretreatment with bisoprolol decreased the serum level of TNF-α compared with the control diseased group. These results

coped with **Liu et al. [37]**, who showed that TNF-α serum level was reduced in both doses of bisoprolol (2 mg/kg and 8 mg/kg) compared to cadmium-induced cardiotoxic group in rats. Moreover, **Zhang et al. [38]** demonstrated that myocardial I/R injury increased the expression of TNF-α, but it was markedly reduced by the bisoprolol treatment.

According to the current findings, myocardial I/R damage significantly raised lipid peroxidation markers, as seen by the control group's higher MDA contents than those of the sham-operated group. This outcome is explained by the fact that I/R injury produces free radicals formed from oxygen, which lead to lipid peroxidation and may also induces further tissue damage, such as cardiomyocyte apoptosis. One of the byproducts of lipid peroxidation is MDA, which is recognized as a sign of increasing systemic oxidative stress caused by ROS-mediated lipid peroxidation of cell membranes **[19].**

Following myocardial I/R injury, a significant decrease in the antioxidant enzyme's activities was observed, as evidenced by serum level of SOD that was lower than that of the sham operated group. This finding confirmed the damage of cardiomyocytes during I/R and demonstrated the harm that I/R injury induced to the endogenous antioxidant system. This could be the result of an overabundance of superoxide anions forming, and a reduction in SOD activity that leads to reduction in the elimination of superoxide anions, which could be detrimental to the heart **[3].** Through its ability to convert the superoxide anion radical (O^{-}) into hydrogen peroxide (H_2O_2), which in turn can be turned into oxygen and water, SOD is the first line of defense for the biological system. It is essential for protecting biological system cells from the harmful side effects of reactive oxygen species (ROS) **[39].**

Additionally, the present investigation demonstrated that the control diseased group's serum GSH level was lower than the sham-operated group. One of the body's non-enzymatic antioxidants, GSH maintains cellular redox state to provide an antioxidant defense mechanism against the damaging effects of reactive oxygen species (ROS) **[39].** Thus, it could be regarded as a sensitive indicator of cardiac oxidative stress.

The current study's findings showed that while the serum levels of SOD and GSH in ivabradine-pretreated rats were significantly higher than those of the control diseased and bisoprolol-pretreated groups, the MDA level in rats given ivabradine was lower than its level in those groups.

These findings are in line with that obtained by **Al-Kuraishy et al. [36]** who showed that MDA serum level was reduced in both doses of ivabradine (5 mg/kg and 10 mg/kg) compared to doxorubicin-induced cardiotoxic group in albino male mice. In agreement with the present findings **Ma et al. [40]** in a study conducted to evaluate the effects of ivabradine on myocardial fibrosis in rats with chronic heart failure and demonstrated that SOD and GSH levels were increased in myocardial tissue of the ivabradine group.

The results of the present study revealed that the serum level of MDA in rats pretreated with bisoprolol was decreased, while the serum level of SOD, and GSH increased. In accordance with these findings, **Salehi et al. [41]** showed that I/R increased MDA level. Pre-treatment with bisoprolol (5 mg/kg), which was started 7 days before induction of I/R, decreased MDA levels in the bisoprolol-treated group in comparison to I/R group.

Additionally, in this study the heart was

stained with TTC after I/R injury to detect the size of the infarcted tissue. Since necrotic tissue lacked active enzymes, it remained unstained, but viable myocardium, which had dehydrogenases, reacted with TTC and was stained brick red **[42].** TTC staining is a rapid, easy and spot technique to confirm myocardial infarction **[43].**

Oral pretreatment with ivabradine decreased the percentage of the infarct size as compared to control diseased group. In the bisoprolol-pretreated group, the infarct size of the left ventricle was lower than that of control diseased group but higher than ivabradine-pretreated group. These findings confirmed the protective effect against I\R injury of both drugs. These results were in accordance with studies done by **Dai et al. [28]** and **Mahmoud et al. [3]** who reported that cardiac infarct tissue was detected using TTC staining after MI. They reported that ivabradine reduced the myocardial infarct size. In addition,**Hendawy et al. [34]**reported that ivabradine and metoprolol decreased the size of infarct area compared to MI group.

In the present work, histopathological examination of cardiac tissue was done. H and E-stained tissues showed foci of necrotic muscle and dilated congested blood vessels in control diseased group comparing to sham operated group, where cardiac tissue showing normal appearing cardiac muscle with longitudinal cut section and intact blood vessels. Oral pretreatment with ivabradine led to improvement of muscle necrosis and marked improvement in vascular congestion, while pretreatment with bisoprolol indicating mild improvement of muscle necrosis and improvement of vascular congestion but not the dilatation.

In accordance with our research, **Hendawy et al. [34]**reported that H&E-stained sections in ivabradine (10 mg/kg) treated rats showed small areas of degeneration and deeply stained small pyknotic nuclei in the wall of the left ventricle, with less congested thickened small coronaries. These results are in line with that obtained by **Liu et al.[37],** who reported similar histopathological finding with bisoprolol administration (2mg/kg) in cardiotoxicity induced in rats.

In addition to the previous signs detected by H&E staining, our results of control diseased group revealed strong and marked BAX immunohistochemical stain expression in the cardiac tissue indicating marked cardiac muscle apoptosis, compared to sham operated group cardiac tissue that shows negative BAX immunohistochemical stain indicating no cardiac muscle apoptosis. This result is consistent with that obtained by **Babu et al**. **[44]**, who reported that there was no immunoreactivity in the sham group. Bax immunostaining was evident, where many myocytes had already died in rat model of I/R. Also, **Wang et al.[45]** showed that Bax protein expression increased significantly in myocardial cells with myocardial ischemia/reperfusion, suggesting that myocardial ischemia/reperfusion stimulated myocardial cell apoptosis by upregulating Bax protein expression in myocardial cells of rats. Oral pretreatment with ivabradine showed negative BAX immunohistochemical stain indicating good improvement of cardiac muscle apoptosis. While oral pretreatment with bisoprolol showed weak cytoplasmic BAX immunohistochemical stain indicating mild improvement of cardiac muscle apoptosis.

These results are in alignment with that obtained by **Li-Sha et al.[46]** who demonstrated that the expression levels of BAX in diseased group were significantly

higher than those in normal control group and were apparently reduced after treatment with ivabradine in model of chronic viral myocarditis.

CONCLUSIONS:

Based on earlier research, it can be said that ivabradine protects rats from I/R injury. This is demonstrated by the considerable declines in T-wave voltage, CK-MB, and the percentage of infarct size in each rat. The mechanism of the cardioprotective effect in the present work, is partially due to decreasing the pro-inflammatory cytokines release TNF-α and lipid peroxidation markers MDA and increasing the activity of the antioxidant markers SOD & GSH. This protective effect was higher than that of bisoprolol. The lower efficacy of bisoprolol than ivabradine in the present study may be also attributed to the short duration of treatment (i.e. 6 days) which didn't give enough time for the long-term beneficial effects of bisoprolol such as inhibition of myocardial remodeling. To validate these results, additional experimental and clinical research is necessary.

Declaration of interest

The authors report no conflicts of interest. The authors along are responsible for the content and writing of the paper.

Funding information

None declared

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Citation:

Bahy Eldin, A., khattab, M., Abdelsameea, A., Elgarhi, R. The Possible Cardioprotective Effect of Ivabradine versus Bisoprolol on Myocardial Ischemia/Reperfusion Injury in Rats. *Zagazig University Medical Journal*, 2024; (4521-4540): -. doi: 10.21608/zumj.2024.265340.3135