Research Article

Effect of Sildenafil Citrate against Cardiotoxicity Induced by Isoproterenol in Male Rats

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ABSTRACT

The objective of the study is to investigate cardio protective effect of sildenafil against isoproterenol induce cardiotoxicity. Adult male albino rats of Wistar strain were used in the present study. The animals were divided into a control group and two experimental groups containing twelve rats each. The animals were pretreated with a solution of sildenafil citrate dissolved in distilled water for 21 days before induction of cardiotoxicity using isoproterenol. And the third group treated with isoproterenol alone for two consecutive days. The levels of cardiac enzymes CK-MB, cardiac troponin I, total antioxidant capacity, B-type natriuretic peptide serum level were assisting using ELISA technology. It was find that animals treated with isoproterenol showed a significant increase in cardiac troponin I, CK-MB, B-type natriuretic peptide, total antioxidant capacity when compared with control group. Pretreated sildenafil group has been show that a significant decrease in all previous serum markers when compared with isoproterenol group. This study shows that sildenafil citrate has a potential cardio protective effect through significant decrease in serum level of the heart when compared with intoxicated myocardium.

Keywords: Sildenafil, cardioprotective, isoproterenol, Phosphodiesterase 5, cardiac markers.

INTRODUCTION

Phosphodiesterases (PDEs) are the only known enzymes to degrade intracellular cyclic AMP and/or cyclic GMP. The PDE superfamily consists of 11 families (PDE1- PDE11), each of which has 1 to 4 subtypes [1] Cyclic nucleotide Phosphodiesterases play a critical role in intracellular signalling by selectively hydrolysing the second messenger's cAMP and cGMP that control cAMP- and cGMP-regulated proteins and transcription factors[2] In many pathologies including inflammation, cardiovascular diseases, neurodegenerations and cancer, alterations of intracellular signalling related to PDE isozyme deregulation might contribute in explaining the difficulties observed in the prevention and treatment of these pathologies. The multiplicity of biochemical, structural properties, their specific subcellular compartments, transcriptional and posttranscriptional regulation make possible to envision to target only the altered PDE isozymes, thus avoiding and/or decreasing the adverse effects induced by non-specific treatments [2] Sildenafil was the first of the PDE5i medicines to be established. It is a ten times more selective for PDE5 than PDE6 and is 1000 times further selective for PDE5 than PDE2-4 [3] Phosphodiesterase-5 inhibitors (PDE5Is) increase erectile function by augmenting nitric oxide availability in the penis and it's providing vasculature, causing in vasodilation and increased blood flow [4] Nitric oxide ($NO^{\cdot}$) stimulates soluble guanylyl cyclase (GC) to yield cyclic guanosine monophosphate (cGMP), cGMP exerts its physiological action over cGMP-dependent protein kinase G (PKG) [5] cGMP stimulation of PKG in cardiomyocytes depresses cellular calcium, which can decrease contractility and counter calcineurin-activation dephosphorylation/stimulation/nuclear translocation of NFAT (nuclear factor of activated T cells), which stimulates expression of a cadre of prohypertrophic genes. Protein kinase G-mediated phosphorylation of an unknown protein increases opening of mitochondrial K+/ATP channels, so diminishing damages causing from ischemia/reperfusion or myocardial infarction. Furthermore, cGMP elevation will suppress β-adrenergic signalling in myocardium and will be associated with activation of PKG phosphorylation of troponin I [6] Calcium plays a main role in the regulation of contractility, growth and gene expression in cardiac myocytes. Most important major function of Ca 2+is to permit excitation–contraction coupling [7] Sildenafil is an effective inhibitor of phosphodiesterase 5 PDE-5, an enzyme which lowers the cellular messenger cGMP. cGMP is play an important role in reducing vascular tone and is a crucial mediator of the effects of natriuretic peptides and nitric oxide. cGMP as well regulates cardiac function, and its production in response to nitric oxide or natriuretic peptides in countering adrenergic stimulation and reduces the development of hypertrophied myocardium. [8]

Phosphodiesterase 5 Inhibitors in Cardio protection:

Since first report showing the cardio protective effect of sildenafil in 2002, there has been tremendous growth of preclinical and clinical studies on the use of PDE5 inhibitors for cardiovascular diseases and cancer. Numerous animal studies have demonstrated that PDE5 inhibitors have powerful protective effect against [10]:

- Myocardial ischemia/reperfusion (I/R) injury
- Doxorubicin cardio toxicity
- Diabetic cardiomyopathy
- Cardiac hypertrophy
- Duchenne muscular dystrophy
- The improvement stem cell efficacy for myocardial repair

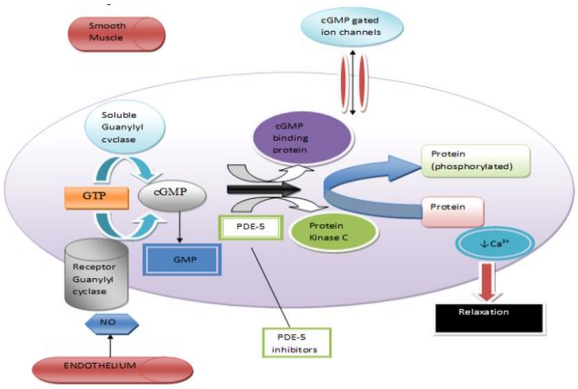

Figure 1 [9]**:** Endothelial molecular pathways controlling NO activity and smooth muscle relaxation and site of action of PDE5 inhibitors. No nitric oxide, cGMP cyclic guanosine monophosphate, GMP guanosine monophosphate, PDE-5 phosphodiesterase 5, GTP guanosine triphosphate.

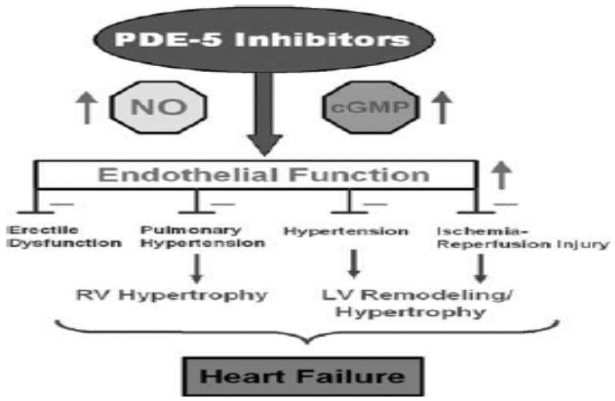

Figure 2[11]**:** Cardiovascular protection with PDE-5 inhibitors. Abbreviations: RV, right ventricle; LV, left ventricle.

Biochemical markers in cardiac diseases

Cardiac troponin

Serum troponin-I is a sensitive indicator of myocardial damage but abnormal troponin I levels have been also reported without acute coronary syndrome and without cardiac damage. Cardiac troponin I is released from myocytes in both reversible and irreversible myocardial injury. The changes in myocyte membrane permeability resulting from the injury could be enough for the release of cardiac troponins from the free cytosolic pool of myocytes without structural damage [12] Pathobiological classification of types of potential mechanisms causing troponin elevations [13]:

Type 1:	Myocytes necrosis
Type 2:	Apoptosis
Type 3:	Normal myocyte turnover
Type 4:	Cellular release of proteolytic troponin degradation products
Type 5:	Increased cellular wall permeability
Type 6:	Formation and release of membranous blebs

Creatine kinase-MB

Commonly available tests to assess cardiac injury include creatine kinase (CK) and CK isoenzymes**.** Classically, an increase in the myocardial-specific enzyme CK-MB is considered the hallmark of acute myocardial infarction, and increased levels are frequently interpreted by the clinician as objective evidence of myocardial cell damage [14], serum total CK activity and CK-MB concentration rise in parallel following myocardial injury, starting to increase 4±6 hr after injury, reaching peak serum concentrations after 12±24 hr and returning to baseline after 48±72 hr Serum CK-MB is considerably more specific for myocardial damage than is serum total CK, which may be elevated in many conditions where skeletal muscle is damaged [15].

B-type natriuretic peptide (BNP)

Natriuretic peptide (NP) levels (B-type natriuretic peptide (BNP) and N-terminal pro BNP) are now widely used in clinical practice and cardiovascular research throughout the world and have been incorporated into most national and international cardiovascular guidelines for heart failure. Researchers was prove that , the release of BNP results in improved myocardial relaxation and serves an important regulatory role in response to acute increases in ventricular volume by opposing the vasoconstriction, sodium retention, and antidiuretic effects of the activated renin-angiotensin-aldosterone system [16].

MATERIALS AND METHODS

Animals and study design

Thirty six adult male Wister rats (weighing 200–250 gm) were used in the experiment. They were obtained from the animal house at the national center for drug and research. Animals were divided into 3 groups randomly each group contains 12 animals, **group A** which received oral saline as –ve control by using oral gavage tube for 21 days, **group B** received isoproterenol (150mg/kg) subcutaneously for two consecutive days to induce myocardial injury, **group C** received sildenafil (10 mg/kg/day) oral solution by using oral gavage tube for21 days in addition to isoproterenol (150 mg/kg) , subcutaneously on the 20th and 21st day from starting sildenafil for 21 days, 24 hours, from the last dose of Isoprenaline, rat will be sacrificed and the level of cardiac marker enzymes will be After assessed in serum.

Plastic cages of (20x25x35 cm) dimension used to keep three animals per cage. Animals within the same cage were differentiated by tail markings using a waterproof

marker. Before starting study protocol the animals were kept for 2 weeks under controlled conditions of temperature of (22 ± 1^0C) with light schedule of 12-12 hour's light/dark cycles and the animal house was provided with an air vacuum to be adapted with the environment of the animal house. Foods commercial pellets and water were accessible freely to the animals.

Treatment administration

Each day of the experiment sildenafil citrate oral stock solution (3.5mg/ml) is prepared freshly by dissolving (50mg) white to off-white sildenafil citrate crystalline powder in 14.3 ml distilled water. A calculated dose of sildenafil for each rat (in treatment group) was drawn in an insulin syringe then delivered to the animal via direct instillation into the stomach or lower esophagus(gavage) of a conscious rat is by far the most accurate method to administer drugs into the gastrointestinal tract . Using adequate manual restraint, the rat was held in a vertical position with its nose aimed toward the ceiling so as to form a straight line between the rat's mouth and stomach. Next, the gavage needle was gently inserted into the oral cavity through the left diastema and passed along the roof of the oral cavity toward the ramus of the right mandible. As the animal swallows, the instrument was advanced down into the esophagus. Finally, once the needle was advanced to the appropriate depth, the solution slowly infused by depressing the plunger of the syringe.

Animal sacrifice

At the end of 22 days, blood collected from animals by cardiac puncture then rats euthanized by decapitation.[17]

Serum sample preparation

Collected blood was allowed to clot for 30 min in plane tube without an anticoagulant at 25°C. Then, blood samples were centrifuged at 2,000 x g for 15 min, and serum layers were pipetted off without disturbing the white buffy layers. Fresh serum is directly use to detect total antioxidant capacity (TAC).Serum were stored in refrigerator at (2-8) C° for detection of troponin I. Subsequently, serums were stored in frozen state for detection of B-type natriuretic peptide and creatine kinase -MB.

Detection of serum rat biomarkers (troponin I, creatine kinase –MB, B-type natriuretic peptide, total antioxidant capacity):

All kits is based on sandwich enzyme –linked immunosorbent assay (ELISA) technology. Anti-biomarkers antibody is pre-coated onto 96-well plates. Standards or samples are added to the appropriate micro ELISA plate wells and combined with specific antibody. Then a biotinylated detection antibody and avidin-horseradish peroxidase (HRP) conjugate is added to each micro plate well and incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce blue color product that change into yellow after adding acidic stop solution. The density of yellow is proportional to the amount of biomarker sample captured in plate. The optical density OD is measured spectrophotometrically at 450nm in microplate reader, then the concentration of biomarkers can be calculated in the sample by comparing the OD of the samples to the standard curve.

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-16.0 (Statistical Packages for Social Sciences- version 16) for windows (SPSS Inc, Chicago, IL). Data were presented in simple measures of mean± standard deviation. The significance of difference of different means were tested using **ANOVA** test for difference among more than two independent means followed by Tukey test. Statistical significance was considered whenever the P value was less than 0.05 and highly significant if it was less than 0.01

RESULTS

Serum cardiac troponin I level

The descriptive statistics, which represent as mean ± standard deviation for cardiac troponin I concentration was significantly higher in the ISO group compared with the control group (171.08 ± 4.05 vs 88.67 ± 3.94 pg/ml, respectively; P < 0.05). The cardiac troponin I concentration was significantly lower in the sildenafil + ISO group compared with the ISO group (135.42 ± 3.99 pg/ml; P < 0.05).as shown in the figure (3-1).

All group when compared to control group (group A) using ANOVA test, had statistical significant where P= 0.05.

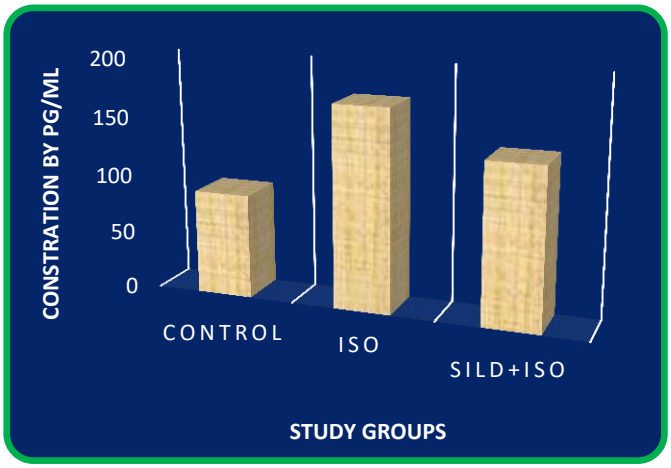

Figure 3: Serum cardiac troponin I level among the studied groups.

Serum Cardiac enzyme CK-MB level

The descriptive statistics, which represent as mean ± standard deviation for cardiac enzyme CK-MB concentration was significantly higher in the ISO group compared with the control group (3.11 ± 0.06 vs 2.51 ±

0.04 unit/ml, respectively; P < 0.05). The cardiac enzyme CK-MB concentration was significantly lower in the sildenafil + ISO group compared with the ISO group (2.70 ± 0.09 unit/ml; P < 0.05).as shown in the figure (3-2).

All group when compared to control group (group A) using ANOVA test, had statistical significant where P= 0.05

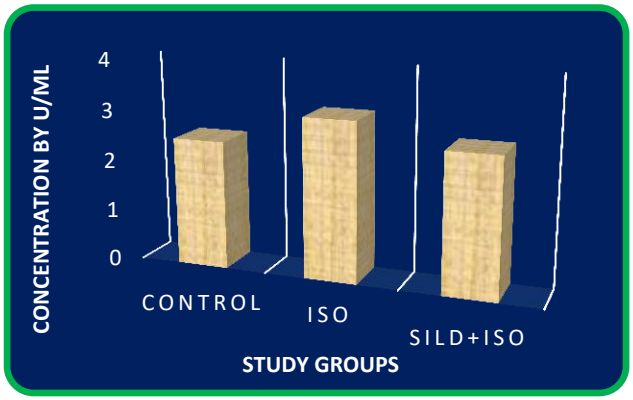

Figure 3-2: Serum Cardiac enzyme CK-MB level among the studied groups.

Brain natriuretic peptide level: Serum

The descriptive statistics, which represent as mean ± standard deviation for Brain natriuretic peptide concentration was significantly higher in the ISO group compared with the control group (56.81 ± 4.69 vs 37.85 ± 2.42 pg/ml, respectively; P < 0.05). The Brain natriuretic peptide concentration was significantly lower in the sildenafil + ISO group compared with the ISO group (44.81 ± 2.29 pg/ml; P < 0.05).as shown in the figure (3-3)

All group when compared to control group (group A) using ANOVA test, had statistical significant where P= 0.05

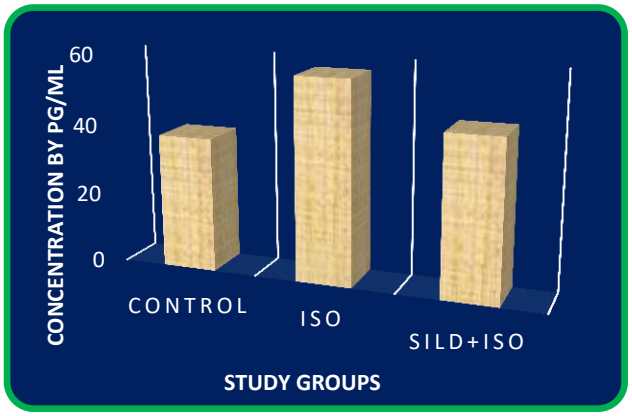

Figure 3-3: Serum Brain natriuretic peptide level among the studied groups

Serum total antioxidant capacity level

The descriptive statistics, which represent as mean ± standard deviation for total antioxidant capacity(TAC) concentration was significantly higher in the ISO group compared with the control group (1.54 ± 0.03 vs 1.25 ± 0.02 unit/ml, respectively; P < 0.05). The total antioxidant capacity was significantly lower in the sildenafil + ISO group compared with the ISO group (1.36 ± 0.02 unit/ml; P < 0.05).as shown in the figure (3-4).

All group when compared to control group (group A) using ANOVA test, had statistical significant where P= 0.05.

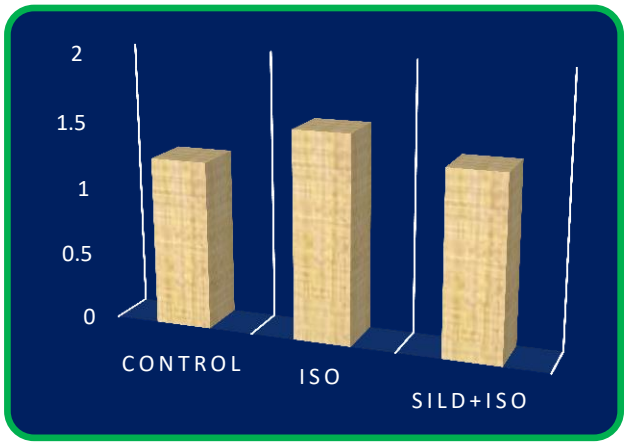

Figure 3-4: Serum total antioxidant capacity level among the studied groups

DISCUSSION

Changes in Cardiac troponin I

Cardiac troponin I (cTnI), C (cTnC) and T (cTnT) are definitive biomarkers for detection of myocardial injury in humans and have proven utility in preclinical studies for drug-induced cardiac injury in animals. Increases in serum cTn also correlate with morphological changes in the heart [18] These contractile proteins are released from myocardium in proportion to the degree of tissue injury and disruption of myocyte membranes.

The published case reports and studies in animals and persons showing that; the effectiveness of serum cTnI in detection of cardiac injury with ischaemic heart disease, cardio- myopathy, heart failure, cardiac contusion, symptomatic cardiac effusion, valvulopathy, cardiac arrhythmias, and prolonged, intense, and unaccustomed exercise [19] ISO is a synthetic catecholamine and undergoes rapid metabolism, resulting in a very short duration of action, also, ISO is a very potent β agonist. ISO has become widely used in toxicological studies as a model drug to induce cardiac muscle injury with myocardial ischemia and the formation of infarct-like lesions. The mechanism of toxicity is therefore closely related to the pharmacological action of the drug. In the rat, at high doses, ISO quickly stimulates β_1 and β_2 receptors in the heart, inducing an abnormally rapid heart rate (β_1 activity) and a fall in blood pressure (β_2 activity), resulting in cardiac tissue anoxia/hypoxia due to elevated oxygen demand and bringing about severe myocardial necrosis [20] In this study, an significant increased level of serum cTnI in ISO-treated rats was observed relative to the control group. The increased level of cTnI may be attributed to the ISO-induced cardiac damage. Animals treated with ISO following pretreatment with sildenafil,

however, exhibited a significant reduction in cTnI levels when compared to ISO-treated rats without sildenafil pretreatment. Our results are consistent with those from a previous study reported by Mahmut Acikel [21]. Pretreatment with sildenafil significantly decreased serum cTnI levels in ISO-treated cardio toxic rats. It is assumed that sildenafil may preserve the structural and functional integrity of the contractile apparatus, which prevents cardiac damage and leakage of troponins from the heart into the blood.

Changes in Cardiac enzyme CK-MB

The myocardium contains high concentrations of diagnostic markers of MI; once it is metabolically damaged, it releases its contents into the extracellular fluids [22] Of all the macromolecules leaked from the damaged tissue, myocardial enzymes are the best markers of tissue damage because of their tissue specificity and catalytic activity. When myocardial cells are damaged or destroyed due to a deficiency in the oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture entirely, resulting in the leakage of enzymes [23] The activity assay for CK-MB in serum is an important diagnosis because of the marked abundance of this enzyme in myocardial tissue and its virtual absence from most other tissues and its consequent sensitivity. CK-MB isoenzyme activity is useful as an index for the early diagnosis of not only myocardial infarction, but also any type of myocardial injury. Leakage of cytosolic enzymes including CK-MB, LDH, AST, and ALT (which serve as diagnostic markers from the damaged tissue) into the blood stream may occur when cell membranes become more permeable or rupture. The amounts of these cellular enzymes in the serum reflect the alterations in plasma membrane integrity and/or permeability [24] Furthermore, the amount of the enzymes appearing in serum is reported to be proportional to the number of necrotic cells , which also reflects a nonspecific alteration in the plasma membrane integrity and/or permeability as a response to *β*-adrenergic stimulation [25] In the present study, rats administered with ISO showed significant increases in the levels of CK-MB enzyme in serum, in line with the results from previous reports, indicating ISO-induced necrotic damage of the myocardium and leakiness of the plasma membrane[21, 23,26)] Pretreatment with sildenafil, however, resulted in lowered activities of CK-MB enzyme in the serum, indicating that sildenafil helps in maintaining the membrane integrity, thereby restricting the leakage of this enzyme.

Changes in brain natriuretic peptide

The natriuretic peptide system (atrial natriuretic peptide, brain natriuretic peptide (BNP) and C natriuretic peptide) is an important marker of cardiac failure. These peptides are synthesized in atrial or ventricular myocytes in response to wall tension [27] To counteract volume overload, biological actions of BNP include natriuresis, vasodilation, and suppression of sympathetic activity [28] Moreover, plasma BNP and NT-proBNP levels are increased in heart failure and aid its diagnosis. These peptide levels provide prognostic information in patients with heart failure and guide the optimization of heart failure therapy [29] In previous studies, serum BNP levels were shown to increase in ISO-induced MI models [30], In this study, an significant increased level of serum BNP in ISO-treated rats was observed relative to the control group. The increased level of BNP may be attributed to the ISO-induced cardiac damage. Animals treated with ISO following pretreatment with sildenafil, however, exhibited a significant reduction in BNP levels when compared to ISO-treated rats without sildenafil pretreatment. Our results are consistent with those from a previous study reported by Zhang W [31] and inconsistent consistent with those from a previous study reported by Ling liu [32] .

Changes in Total antioxidant capacity

Isoproterenol (ISO), a β-adrenergic agonist, is identified to produce cardiac ischemia due to free radical production by autoxidation [33] Auto-oxidation of ISO produces quinones, which react with oxygen to produce superoxide anions and hydrogen peroxide, causing oxidative stress and depletion of the endogenous antioxidant system. Free radical scavenging enzymes, such as SOD, CAT, GPx, GR and GST are initial line of cellular defense against oxidative injury [34] There are three categories of antioxidant species: enzyme systems (GSH reductase, catalase, peroxidase, etc.), small molecules (ascorbate, uric acid, GSH, vitamin E, etc.) and proteins (albumin, transferrin, etc.). Different antioxidants vary in their reducing power [35] The 'Total antioxidant capacity' (TAC) is a parameter frequently used for characterization of food products and of the antioxidant status of the body. The term TAC is not optimal since the assay methods only part of antioxidant capacity, typically excluding enzymatic activities [36] It has been described that different antioxidants can be produced depending on which ROS are formed. Plasma antioxidant status can therefore vary according to which antioxidant is quantified(37) . In this study the results showed that the level of total antioxidant capacity was, paradoxically, significantly increase in group B which take isoproterenol 150 mg/kg for two consecutive days when compared to control group This findings totally agree with Gawron A, *et al*, which find that "The level of total antioxidant capacity estimated with FRAP method was higher in men with coronary heart disease in comparison with healthy men" [38] and agree with Savu O, *et al*, which found that : " Increase in total antioxidant capacity of plasma despite high levels of oxidative stress" [37] while the total antioxidant capacity level was significantly decrease in group C, sildenafil citrate pretreated group, when compared with isoproterenol group as a result of its remarkable decrease of reactive oxygen species production [39].

CONCLUSIONS

1. Administration of isoproterenol subcutaneous may yield a model of cardiotoxicity disease indicated by cardiac damage which in turn improved by sildenafil treatment.
2. Present study demonstrates that sildenafil citrate exerts its cardioprotective effects.
3. Sildenafil citrate has a potential role in decreasing oxidative stress.

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