Bull. Egypt. Soc. Physiol. Sci. *40(2)*, 16-30

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Effect of Abscisic Acid on Isoproterenol-Induced Myocardial Infarction in Rats: A Possible Role of Nitric Oxide

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Received: 30 Sept 2019
Accepted: 25 Nov 2019
Available online: 1 July 2020

Keywords

- Abscisic acid
- Isoproterenol
- Myocardial Infarction
- Nitric oxide

Abstract

The aim of this study was to find out the possible role of nitric oxide (NO) as a second messenger in stress responses in the cardioprotective effect of the abscisic acid (ABA) on isoproterenol (ISO)-induced myocardial infarction (MI) in rats. **Material and method**: Thirty male adult rats distributed into: normal control group, MI group, and MI group treated with ABA (MI+ABA) for one week. The cardiac effect of ABA on ISO-induced MI was evaluated by electrocardiogram (ECG), measuring of mean arterial pressure (MAP), markers of cardiac injury in the serum, the cardiac content of antioxidants and lipid peroxidation products, observing myocardial pathological changes, indirect detection of NO was performed and the myocardial content of the inducible nitric oxide synthase (iNOS) was determined by ELISA. **Results:** Compared with the MI group, we found that ABA could ameliorated ISO-induced disturbances in the ECG pattern and in MAP. Moreover, the concentration of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), troponin I (cTn-I), nitrite and nitrate were significantly decreased in the MI+ABA group. The contents of catalase (CAT), and reduced glutathione (GSH) were elevated. Meanwhile, the level of malondialdehyde (MDA) and iNOS were reduced with ABA. In addition, the histopathological examination showed that the pathological changes in the myocardium that observed in the MI group partially improved in the group treated with ABA. **Conclusion:** Our findings suggest that ABA has cardioprotective effects against the harmful effects induced by ISO that may be mediated by its antioxidant properties and prevention of the cytotoxic actions of the excessive release of NO via inhibition of iNOS.

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INTRODUCTION

Ischemic heart disease is one of the most widely spread cardiovascular disease which include angina pectoris, ischemic heart failure, arrhythmia, and myocardial infarction. Myocardial infarction (MI) is an acute disorder characterized by myocardial necrosis, which is associated with the disparity between myocardial demand and coronary blood supply (1). Earlier studies have demonstrated that MI results from a complex set of pathological processes, together with an inflammatory cell infiltration, increase formation of free radical, irreversible damage of DNA and apoptosis (2). Medical treatments of MI are palliative and its clinical manifestations improved by increasing perfusion and decreasing myocardial oxygen consumption. Moreover, the effects of these treatments are limited, and associated with harmful side effects. Therefore, it is needed to search for new compound with lower toxicity and more effective for the prevention and treatment of MI.

Abscisic acid (ABA) is a phytohormone involved in the physiological process in higher plants (3). ABA usually called the 'stress hormone' which controls many features of plant growth and development under stressful conditions such as (temperature, water and nutrient availability, light). When exposed to chemical or physical stress, human leukocytes produce ABA, which improves phagocytic activity of leukocytes (4). In human, ABA also released from adipose tissue and from β-cells of pancreas in response to glucose (13). Moreover, high levels of ABA were noticed in mesenchymal stem cells in the bone marrow (6). Chaqour et al. (7) found that ABA prevents neovascularization by suppressing the angiogenic phenotypes of endothelial cells and macrophages, indicating its possible role as antiangiogenic therapy.

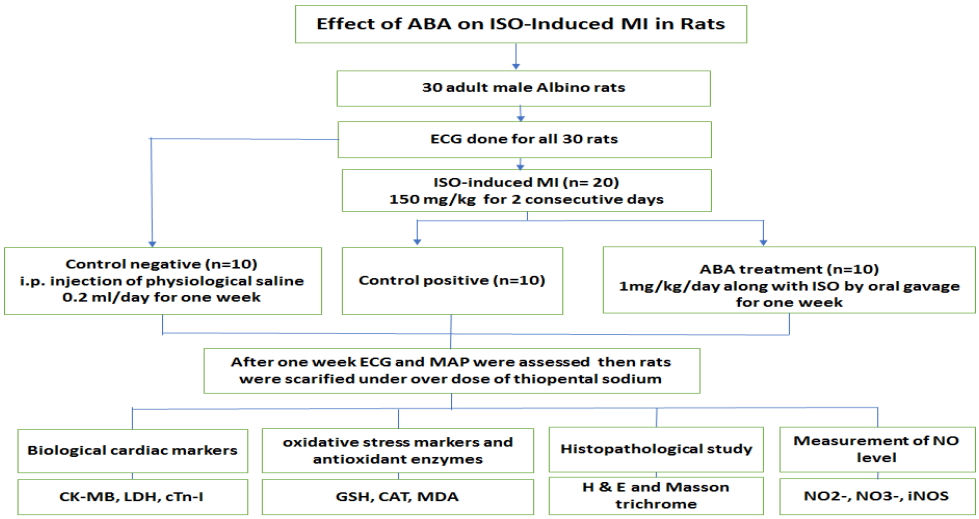
Nitric oxide (NO) is a short life bioactive molecule that considered as a toxic compound, but later on NO were described to be an important signal and an effector molecule in cell physiology (8). In mammalian, NO is synthesized endogenously by converting L-arginine into L-citrulline, this process catalyzed by nitric oxide synthase (NOS) enzyme. There are three different isoforms of the NOS, which are referred to as neuronal NOS (nNOS or NOS I), inducible NOS (iNOS or NOS II), and endothelial NOS (eNOS or NOS III) (9). At the level of cardiovascular system, NO is considered as a vital intracellular biological active molecule that have different physiological and pathophysiological functions, including regulation of cardiac contractility and diameter of blood vessels (10). However, the effect of NO in the myocardial damage and dysfunction that occur during ischemia reperfusion remains unclear. Increase expression of iNOS during myocardial ischemia produces excess amount of NO which accompanied with increased production of reactive oxygen species (ROS), including superoxide and peroxynitrite ($OONO^-$), which are harmful to the heart (11). Aktan (12) revealed that the level of iNOS was positively correlate with the expression of pro-inflammatory cytokines and severity of cardiac dysfunction. On the other hand, endogenous NO may play an important role in initiation of ischemic preconditioning protection (13). Moreover, several previous studies revealed that administration of NO or NO donors before ischemia diminishes the consequences of myocardial ischemia reperfusion, together with reduction of infarct size and endothelial dysfunction (14,15).

The hypothesis of our study deduced from an in-vitro study of Vigliarolo et al. (16) Whose suggested the protective role of endogenous ABA against oxygen depletion in cardiomyocytes through modulation of NO production and increase glucose uptake. Our aim in the current study was to demonstrate the protective effect of ABA on ISO-induced MI in rats, and exploring its mechanism that based on the antioxidant activity of ABA and possible involvement of NO.

Material and methods:

Thirty male Albino rats (200±10g) were purchased from Medical experimental research center (MERC), Faculty of Medicine, Mansoura University, Egypt and allowed to acclimatize for one week before starting the study in facilities, where the room temperature was controlled at 24±5 and 45 to 55% humidity with a regular 12h light–dark cycle. During the entire experimental period all rats have free access to water and standard rat pellet diet. Our Local Committee of Animal Care and Used approved this protocol (Code number: R.19.10.658).

After acclimatization, the rats were randomly divided into three groups, each one contains 10 rats: normal control group (C), the normal rats injected intraperitoneally, i.p. with physiological saline 0.2 ml/day for one week), MI group; the rats of this group injected with 150 mg/kg of isoproterenol (ISO) for 2 consecutive days to induce MI (17), MI+ABA group; MI group treated with ABA, in this group the rats injected with 150 mg/kg of ISO for 2 consecutive days to induce MI+1mg/kg/day of ABA was administered along with ISO by oral gavage for one week. ISO and ABA were obtained from (Sigma Chemical Co., St. Louis, MO, USA). Body weight (BW) and electrocardiogram (ECG) recording were done before induction of MI and at the end of experiment and any alterations in the ECG were documented. Then, the rats anesthetized with overdose of thiopental sodium. Blood samples were rapidly collected from the heart and left to clot at the room temperature for 10 to 15 min then centrifuged at 3,000 RPM for 15 min to separate serum. Serum was stored at -20 ◦C for further analysis. Weight of the heart (HW) was recorded, then the heart/body weight (HW/BW) ratio was calculated and used to evaluate the degree of cardiac hypertrophy. The heart tissues from left ventricles were homogenized for subsequent analysis.



Flow chart demonstrating the experimental study approach

Measurement of mean arterial blood pressure (MAP):

Mean arterial blood pressure (MAP) was measured using the tail-cuff method with the rats under a conscious condition with LE 5001pressure meter (Panlab technology for Bioresearch, Inc., Spain). The cuff was inflated until the pulse signal disappears due to blocking of the caudal artery. Once the signal disappear, the cuff was deflates automatically, the first appearance of the pulse was considered as systolic blood pressure (SBP). Then, the cuff was continues to deflate until the pulse signal return to its initial value; the pressure reading at this moment represent diastolic blood pressure (DBP). Once the values of systolic and diastolic pressure were obtained, the system automatically calculates the MAP by using the following formula: MAP = DBP + 0.33 (SBP - DBP).

Electrocardiogram assessment:

At the start of experimental protocol ECG recording was done for all rats, then another ECG recording was done before killing the animals. ECG recording was done under anesthesia; ketamine hydrochloride (25 mg/kg) plus xylazine (5 mg/kg) (18) using BIOPAC student lab system (software BSL 3.7.5), in the Medical Physiology Department, Faculty of Medicine, Mansoura University, Egypt. ECG leads recorded through skin surface electrodes. The neutral electrode was connected to the right-hand leg, right foreleg was connected to the negative electrode, while, left foreleg was connected to the positive electrode.

Analyses of biological cardiac markers, oxidative stress markers and antioxidant enzymes:

The myocardial cellular injury was assessed by spectrophotometrically measuring serum levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and cardiac troponin-I (cTn-I) according to the manufacturer's instructions (Abx Diagnostics, Montpellier, France). Myocardial tissue was taken from the left ventricle of the rats and kept in -80℃ refrigerator, the activities of reduced glutathione (GSH), catalase (CAT), and contents of malondialdehyde (MDA) in the heart homogenate were evaluated according to the diagnostic kit instructions (Sigma Chemical Co., St. Louis, MO, USA).

Histopathological study:

The samples from left ventricle were preserved in 10% buffered neutral formalin for 24h, then, specimens were prepared in paraffin sections and stained with hematoxylin & eosin (H&E) in order to evaluate the morphological changes. Moreover, Masson trichrome was performed to examine collagen deposition in stained sections. Prepared sections were observed under high power microscope (x200) to evaluate myocardial injury induced by ISO and the effects of ABA treatment, and photomicrographs were taken.

Measurement of nitric oxide level:

Serum level of NO was measured indirectly by the quantification of nitrites ($NO2^-$) and nitrates ($NO3^-$) ions with the Grieiss reagent (19). Moreover, the myocardial content of the iNOS determined by ELISA detection kits and the assays performed according to the manufacturers' instructions (Sigma Chemical Co., St. Louis, MO, USA).

Statistical analysis:

All our descriptive data are presented as the mean ± SD for each dependent variable of ten rats. Data were analyzed using one-way ANOVA followed by post-hoc Tukey's test to evaluate the significance between the different groups using SPSS version 16 (Chicago, IL, USA). Values with P< 0.05 were considered statistically significant.

Results:

The data presented in **Table (1)** revealed a significant (P ≤ 0.001) elevation in HW/BW of MI group in comparison to control one. However, HW/B ratio was significantly (P ≤ 0.001) reduced in MI+ABA group when compared with MI group. Moreover, the MAP was significantly (P ≤ 0.001; **Table1)** decreased in MI rats in comparison to the C group. There was a significant (P ≤ 0.001) increase in MAP in the MI+ABA group as compared with MI group.

The electrocardiogram and related parameters of all rats were presented in **Table (2).** The rats in the C group showed normal patterns of ECG, whereas rats in MI group showed significant (P ≤ 0.001) decrease in QRS-amplitude along with significant (P ≤ 0.001) elevation of ST segment when compared to the normal group, this result indicate occurrence of MI. In comparison to MI group, treatment of infarcted rats with ABA showed significant (P≤ 0.001) decrease in the elevation of ST segment. Furthermore, MI group showed a significant decrease (P ≤ 0.001) in the duration of PR interval, QRS wave and RR intervals as compared to C group. Treatment of MI rats with ABA revealed significant improvement of these changes as compared to rats in MI group.

Table (1): Effect of abscisic acid on body weight (BW), heart weight/ body weight (HW/BW) ratio, and MAP in different experimental groups:

	C	MI	MI+ABA
Body weight (g)	203.17 ± 7.85	157.33 ± 2.16 [a]	174.17 ± 2.23[ab]
HW/BW ratio	1.47 ± 0.03	1.94 ± 0.04 [a]	1.68 ± 0.03[ab]
MAP (mmHg)	80.47 ± 1.8	39.62 ± 1.40 [a]	57.07 ± 2.39[ab]

MAP: Mean arterial blood pressure, C: control group, MI: group treated with isoproterenol only, MI+ABA: myocardial infarction rats treated with abscisic acid. Data were expressed as mean ± SD, test used: One-way ANOVA, followed by post-hoc Tukey's test, P: significance <0.05. [a]: significance as compared to C group. [b]: significance as compared to MI group.

Table (2): ECG parameters in different experimental groups:

	C	MI	MI+ABA
ST segment (mv)	0.174 ± 0.002	0.335 ± 0.003[a]	0.237 ± 0.001[ab]
QRS amplitude (mv)	0.358± 0.011	0.27 ± 0.009[a]	0.308 ± 0.011[ab]
QRS duration (sec)	0.04 ± 0.001	0.029 ± 0.001[a]	0.032 ± 0.001[ab]
PR interval (sec)	0.044 ± 0.002	0.029 ± 0.001[a]	0.037 ± 0.001[ab]
RR interval (sec)	0.255 ± 0.015	0.143 ± 0.012[a]	0.192 ± 0.007[ab]
Heart rate (beat/min)	282.75 ± 2.67	362.27 ± 5.39[a]	321.38 ± 2.62[ab]

C: control group, MI: group treated with isoproterenol only, MI+ABA: myocardial infarction treated with abscisic acid. Data were expressed as mean ± SD, test used: One-way ANOVA, followed by post-hoc Tukey's test, P: significance <0.05. [a]: significance as compared to C group. [b]: significance as compared to MI group.

Regarding to the cardiac markers Figure (1) showed significant (P≤ 0.001) increase in the serum levels of CK-MB, LDH, and cardiac troponin-I in the rats of MI group as compared to C group. While, MI+ABA group showed significant (P≤ 0.001) improvement in their serum levels in comparison to the MI rats. To investigate whether the effect of ABA on MI were associated with oxidative stress or not, we verified the concentration of GSH, CAT, and MDA in the myocardial homogenate. As shown in Figure (2) the content of CAT and GSH were significantly reduced in the MI group in comparison to the rats in C group, meanwhile, the content of MDA was significantly elevated in the MI group in comparison to C group. Interesting, treatment of MI rats with ABA significantly reversed these changes when compared to that in the MI group Figure (2).

The histopathological assessment of H&E-stained heart tissue were scored according to severity of myocardial changes: grade 1 (normal histological appearance of the myocardium), grade 2 (focal necrosis of myocardium), grade 3 (focal necrosis of myocardial fiber with neutrophil infiltration and interstitial edema), and grade 4 (extensive necrosis of myocardial fiber with hemorrhage, interstitial edema, marked neutrophil infiltration and sever myofibrillary degeneration). In the present study, histopathological changes were demonstrated in Figure (3) as follows: in C group, all rats had grade 1; rats in MI group had grade 4; as regard to MI+ABA group, rats had grade 2. Masson's trichrome staining; as demonstrated in Figure (4), assessed cardiac fibrosis in each group: numerous collagenous fibers were appeared in the hearts of rats in the MI group when compared with C group. However, treatment of infarcted rats with ABA, revealed marked decrease in the deposition of collagenous fibers when compared with the MI group.

The data presented in Table (3) revealed a significant (P≤ 0.001) elevation in the serum levels of $NO2^-$ and $NO3^-$ levels in the MI group in comparison to C group. However, significant (p ≤ 0.001) reduction in their level was observed in the MI + ABA group in comparison to MI group. Moreover, the level of iNOS was significantly (P≤ 0.001; Table 3) increased in ISO treated rats (MI group) when compared with C group. There was a significant (P≤ 0.001) decrease in the level of iNOS in MI+ABA group in comparison to MI group.

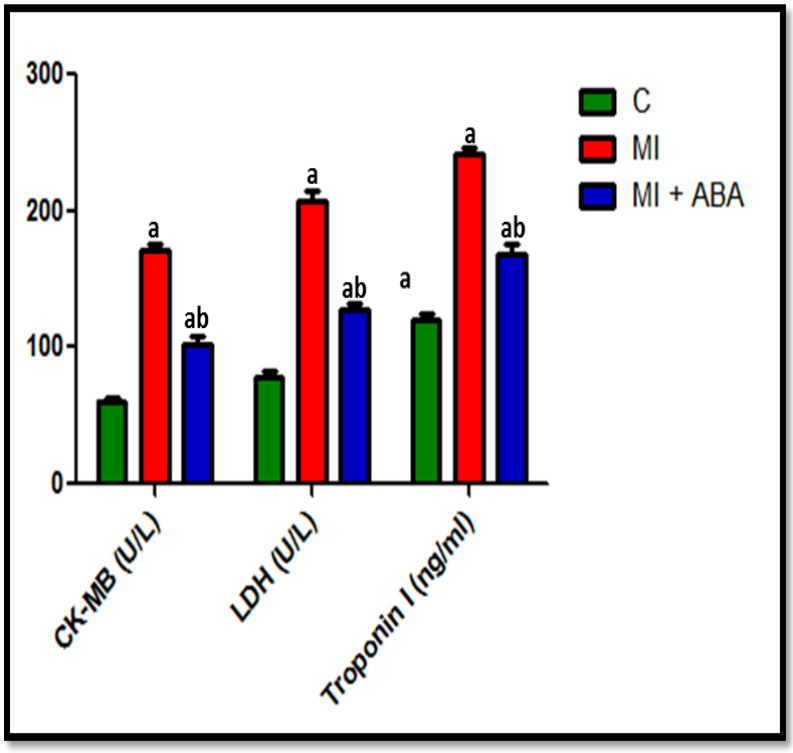


Figure (1): Effect of abscisic acid supplementation on cardiac markers in all experimental groups. CK-MB: creatine kinase MB, LDH: lactate dehydrogenase, C: control group, MI: group treated with isoproterenol only, MI+ABA: myocardial infarction treated with abscisic acid. Data were expressed as mean ± SD, test used: One-way ANOVA, followed by post-hoc Tukey's test, P: significance <0.05. [a]: significance as compared to C group. [b]: significance as compared to MI group.

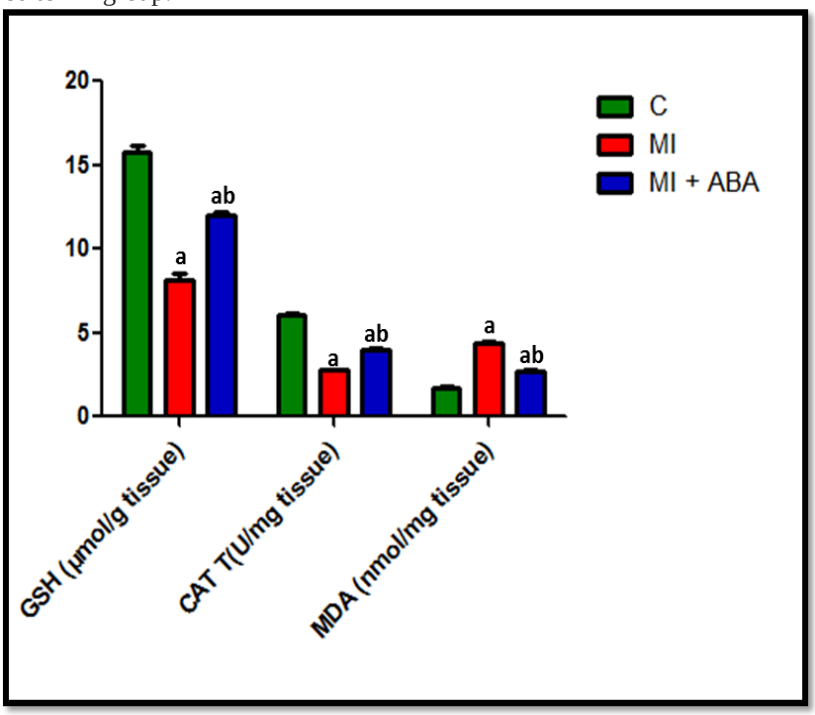


Figure (2): Effect of abscisic acid administration on oxidative and anti-oxidative cardiac markers in all experimental groups. GSH: reduced glutathione, CAT: catalase, MDA: malondialdehyde, C: control group, MI: group treated with isoproterenol only, MI+ABA: myocardial infarction treated with abscisic acid. Data were expressed as mean ± SD, test used: One-way ANOVA, followed by post-hoc Tukey's test, P: significance <0.05. [a]: significance as compared to C group. [b]: significance as compared to MI group.

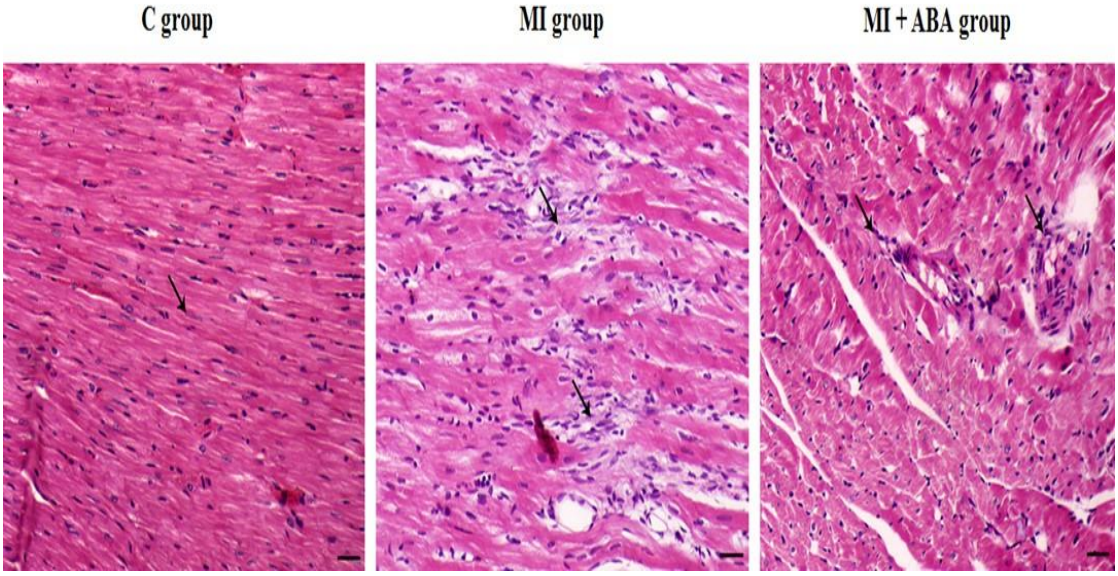


Figure (3): Histopathological examination based on severity of myocardial changes in all experimental groups. Heart of control (C) group-grade 1 showing normal myocardial fibers with cigar-shaped nucleus (arrow). Heart of MI group-grade 4 showing sever myocardial necrosis associated with marked fibrosis, hemorrhage, interstitial edema and marked neutrophil infiltration (arrows).While, the heart of MI+ABA group-grade 2 showing mild degree of interstitial and perivascular fibrosis (arrows). H&E stain, x200, scale bar= 40 µm.

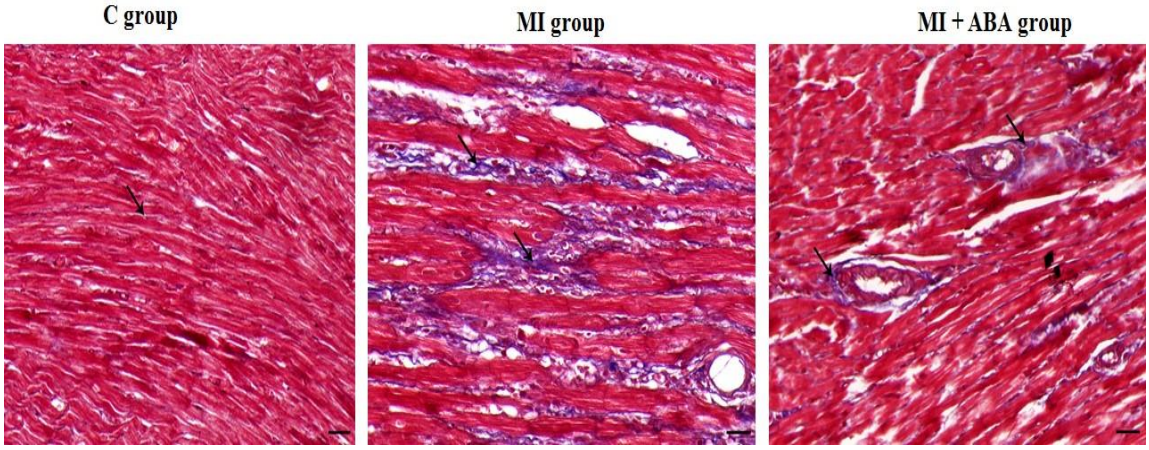


Figure (4): Histopathological examination of the myocardial fibrous tissue. Heart of control (C) group showing normal myocardial fibers separated with thin fibrous layer (arrow). Heart of MI group showing marked fibrosis (arrows). While, the heart of MI+ABA group showing slight fibrosis limited to perivascular areas (arrows). Masson trichrome stain, x200, scale bar= 40 µm.

Table (3): Effect of abscisic acid on the level of $NO3^-$, $NO2^-$ and iNOS in different experimental groups:

	C	MI	MI+ABA
$NO2^-$ (g/mL)	4.75 ± 0.14	7.14 ± 0.19 [a]	5.47 ± 0.14[ab]
$NO3^-$ (g/mL)	17.48 ± 0.18	24.69 ± 0.22 [a]	20.49 ± 0.26[ab]
iNOS (ng/mg tissue)	39.61 ± 1.28	71.92± 3.28 [a]	53.95 ± 1.43[ab]

$NO2^-$: Nitrites, $NO3^-$: Nitrates, iNOS: inducible NOS, C: control group, MI: group treated with isoproterenol only, MI+ABA: myocardial infarction treated with abscisic acid. Data were expressed as mean ± SD, test used: One-way ANOVA, followed by post-hoc Tukey's test, P: significance <0.05. [a]: significance as compared to C group. [b]: significance as compared to MI group.

Discussion:

Myocardial infarction is a pathological condition caused by decreased perfusion of the myocardium. Experimental studies on ISO-induced cardiotoxicity offer a good understanding of this disease. ISO is a synthetic β-adrenoceptor agonist and its injection induces MI in rats (20) as well as ISO diminishes the source of energy in the myocardial cells, which results in permanent cellular damage and infarct-like necrosis (21). Moreover, the acute stage of myocardial damage induced by ISO manifested by changes in ECG, blood pressure, heart rate, and left ventricular dysfunction similar to that occurring in patients with MI (22).

The role of NO in the cardiovascular diseases is complex and controversial. This controversy is perhaps due to the critical balance between NO and one of its metabolic product; peroxynitrite. Under normal physiological conditions, iNOS-derived NO has a cardioprotective effect through its antioxidant properties and vasodilation of blood vessels. However, in response to myocardial ischemia, increased production of iNOS/NO led to the formation of excess amount of peroxynitrite which associated with oxidative stress, that mediate the harmful effects of iNOS/NO (9). Therefore, our hypothesis in the current work is to assess the effects of ABA on ISO-induced MI and involvement of NO in the initiation and severity of MI, which may modulated by administration of ABA.

Our results showed that administration of ISO only, the rats displayed myocardial hypertrophy as supported by the significant increase in HW/BW ratio. These findings were also reported in earlier studies that demonstrated cardiac hypertrophy along with an increase in HW/BW in ISO-administered rats (23,24). This increase in HW/BW ratio is related to a hypertrophic response to ISO that was reported earlier (25). An increase in the HW which noticed in ISO-induced MI may be related to an increase in water content, protein synthesis, inflammatory cells infiltrations and edematous intramuscular space that following extensive necrosis of cardiac muscle (26). Moreover, the observed increased HW in ISO-administered rats may be explained by increased uptake of glucose in the myocardium along with increased oxidative stress (27). We have demonstrated that treatment of ISO-induced MI with ABA significantly decreased the HW/BW which indicating that the ABA attenuate ISO-induced cardiac hypertrophy.

The study of Khorrami et al. (28) reported a decrease of MAP in ISO-induced MI in rats. In addition, several previous works have confirmed decreased pumping ability and contractile activity of the heart in ISO-induced hypertrophy (29,30). In agreement with these previous studies, the results of our work demonstrated a significant decrease in MAP in rats received ISO alone. While treatment of infarcted rats with ABA effectively raises the MAP, this finding indicates the beneficial effects of ABA on myocardial contractility in ISO-induced MI.

ECG is the most significant method for the diagnosis of MI, especially for detection of ST segment elevation (28). In the present work, ISO caused elevation of ST-segment and depression of QRS-amplitude. The elevated ST-segment represent the damage of cell membrane and loss the potential difference between the ischemic and

non-ischemic regions, while depression QRS-amplitude may be due to myocardial edema which induced by ISO (31). In the present study, the rats treated with ISO alone showed a significant decrease in R-R interval, which explain the increased heart rate in this model of MI. It was observed that this increase in heart rate is responsible for augmented oxygen consumption that leads to myocardial necrosis (22). The other characteristic ECG findings of ISO-induced MI in the present study are shortening of the PR interval, QRS complex. The PR segment represents AV conduction where as QRS represents the total duration of ventricular depolarization, their alteration represents abnormality in the functions of the heart (32). Panda et al. (33) found that rapid ventricular depolarization caused by positive inotropic effect of ISO is responsible for this decrease in the duration of QRS in infarcted rats. Administration of ABA significantly restores the ECG pattern near to normal, indicating its protective effects on the cell membrane and electrical discharges, which may be due to its antioxidant property that maintained the integrity and permeability of cellular membranes.

In addition, from the perspective of histopathology, the rats treated with ABA exhibited reduction of cardiomyocyte necrosis, infiltration of inflammatory cells and edema formation compared to rats treated only with ISO. Furthermore, administration of ABA with ISO revealed that deposition of collagen fiber was decreased when compared with rats treated only with ISO. These results suggest that ABA may attenuates effectively the cardiotoxicity caused by ISO and MI-induced fibrosis.

Myocardium contains specific enzymes, and when the myocardial cells undergo damage, these enzymes are released into serum in different degrees, thus measuring the serum level of these enzymes are an important tool in diagnosis and monitor MI (34). Our current study found that serum levels of CK-MB, LDH, and troponin-I were significantly elevated in ISO-treated rats, this result suggesting that ISO-induced myocardial cell damage, which is consistent with previous studies (35). Treatment of ISO-induced MI rats with ABA significantly improved the pathological elevation of these myocardial injury markers, which indicating that the ABA could effectively reduce cardiotoxicity induced by ISO through maintain integrity of cell membrane, thereby reducing the leakage of these enzymes.

Previous studies revealed that, oxidative stress mediated by ROS is one of the mechanisms of ISO-induced cardiotoxicity (36). Once ISO enters the cell, it will generate highly cytotoxic free radicals and produce excessive ROS, resulting in loss of function and integrity of myocardial membranes (37). Moreover, ISO induced damage of heart tissues through modulation of antioxidant system leading to oxidative stress. Furthermore, there is a positive correlation between the degree of oxidative stress and the severity of tissue damage induced by ISO. In general, the intracellular antioxidant enzymes such as GSH and CAT, thereby ensuring a balanced generation and removal of the free radicals (38). When the free radicals accumulate and cannot be removed by antioxidant enzymes, these accumulated free radicals promote the process of lipid peroxidation, which induce cell damage (39). The main product of lipid peroxidation is Malondialdehyde (MDA),

therefore, the tissue content of MDA can reflect the degree of lipid peroxidation and indirectly show the balance between ROS and antioxidant defense system (40). Our results showed that ISO significantly reduce the GSH and CAT content in hearts of rats, which can lead to the accumulation of lipid peroxidation products (MDA) and induce cardiac damage. On the other hand, administration of ABA increased the myocardial content of GSH and CAT and reduced the level of MDA. Therefore, the results of the present study suggest that ABA can reduce the toxicity of heart induced by ISO in rats effectively by improving the antioxidant capacity and ROS scavenging ability, and eventually reducing the degree of oxidative stress and lipid peroxidation.

In order to determine the role of NO in the effect of ABA in ISO-induced MI, the level of NO were measured in serum and iNOS were measured in the myocardium. NO is an extremely unstable molecule and is rapidly converted to $NO2^-$ and $NO3^-$ in vivo and in vitro, therefore serum levels of $NO2^-$ and $NO3^-$ have been used as an index of NO generation (41). In the current study, serum levels of NO were measured indirectly through quantifying its stable products $NO2^-$ and $NO3^-$. According to Table (3), the level of both $NO2^-$ and $NO3^-$ were significantly higher in the MI group than those of control one. The decrease in systolic and diastolic blood pressure caused by ISO explained by Krenek et al. (42); as it could be linked to an increase in NO release. This idea was confirmed in our results that clearly showed marked elevation in the level of both $NO2^-$ and $NO3^-$ accompanied with significant decrease in MAP in rats received ISO only. These results associated with an increase in the activity of iNOS in the group treated with ISO only when compared with control group Table (3). iNOS involved in the synthesis of NO in vivo, the expression of iNOS increased in any condition associated with oxidative stress, resulting in excessive production of NO, this high level of NO easily led to lipid peroxidation. It seems that NO can mediate both protective and harmful myocardial effects. Previous studies revealed that low concentrations of NO improve the function of cardiomyocyte. On the contrary, higher concentrations of NO diminish cardiomyocyte function, worsen mitochondrial respiration, mediate inflammatory response and even induce death of cardiomyocyte (43). On the other hand, administration of ABA decreased both serum levels of $NO2^-$ and $NO3^-$ and myocardial level of ions Table (3). According to these results, the reduction in both $NO2^-$ and $NO3^-$ was related to a decrease in iNOS activity. The reduction in synthesis of NO and inhibition of iNOS in the presence of ABA leads us to the concluded that the administration of ABA contributes to improve heart function and this improvement could be related to modulation of NO synthesis.

Conclusion:

In summary, the present study showed that the ABA could reduce the damage of the myocardium, which induced experimentally by ISO through improving the anti-oxidant capacity and modulation of NO release that may be prevent the cytotoxic effects of the ISO. Therefore, ABA could serve as an important component in curing ischemic heart disease.

Acknowledgements: The Department of Medical Physiology, Faculty of Medicine, Mansoura University and Medical Experimental

Research Center (MERC), Faculty of Medicine, Mansoura University Mansoura University acknowledged for their contribution in the experimental part of the present study.

Author contribution: All authors have accepted responsibility for the whole content of this manuscript and accepted its submission.

Conflict of interest: The authors declare that there is no conflict of interest.

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