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POTENTIAL PROTECTIVE EFFECT OF SITAGLIPTIN AGAINST MYOCARDIAL INFARCTION IN RATS: MITIGATION OF OXIDATIVE STRESS AND INFLAMMATORY MEDIATORS

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ABSTRACT

Background Dipeptidyl peptidase-4 (DPP4) enzyme inhibition has been reported to increase plasma glucagon-like peptide-1 (GLP-1) level for controlling postprandial glucose concentration. DPP4 inhibitors have received attention for the potential of these interventions to positively impact on cardiovascular outcomes. **The aim** of the present study was to explore the potential protective effect of DPP-4 inhibitor on acute myocardial infarction induced experimentally in rats and possible mechanism of action. **Material and methods** Seventy two albino rats were divided randomly into 3 equal groups; normal control group rats were received physiological saline. Myocardial infarction groups pretreated with vehicle or sitagliptin (5mg/kg/day, orally by gastric gavage once a day) for 2 weeks before left coronary artery ligation for induction of acute myocardial infarction model in rats. Four hours after ligation of the left anterior descending coronary artery (LAD), the electrocardiogram, blood pressure, cardiac enzyme (CPK), myocardial tumor necrosis factor alpha (TNF-α), and lipid peroxidation as malondialdehyde level in the cardiac tissue were measured. Additionally, glutathione peroxidase and catalase were determined before and after myocardial infarction in all groups, in addition to histopathological examination. **Results** the induction of myocardial infarction resulted in highly peaked T wave in the ECG tracing, significant increase in serum CPK level, significant elevation of lipid peroxidation and TNF-α content of the myocardial tissue compared with control group. On the other hand, glutathione peroxidase and catalase were decreased. Pretreatment with sitagliptin (5mg/kg/day, orally) 2 weeks before ligation of LAD resulted in a significant decrease in

peaked T wave, insignificant decrease of blood pressure, pronounced reduction in serum CPK level, reduction of myocardium lipid peroxidation and TNF-α content level in myocardial tissues. While, myocardial glutathione peroxidase and catalase contents were significantly increased. Histopathological changes were supported with biochemical changes. **Conclusion** the results highlight the efficacy of sitagliptin as cardioprotective drug against myocardial infarction by improving inflammatory status and oxidative stress.

KEYWORDS: sitagliptin, oxidative stress, TNF-α, myocardial infarction, DPP-4 enzyme inhibitors.

INTRODUCTION

Myocardial infarction (MI) occurs when there is myocardial necrosis due to prolonged imbalance between the myocardial oxygen supply and demand of the myocardium.[1] The accumulations of free radicals have been implicated in the pathophysiology of acute myocardial infarction.[2]

Recently, glucagon-like peptide (GLP)-1 was shown to have cardioprotective effects, but treatment with GLP-1 is limited due to its short half-life. It is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4), an enzyme which inhibits GLP-1 activity.[3] We hypothesized that the DPP-4 inhibitor sitagliptin will increase levels of GLP-1 and may exert protective effects after myocardial infarction.

Sitagliptin is an orally administrated antihyperglycaemic agent that selectively inhibits the dipeptidyl peptidase-4 (DPP-4) enzyme. It prevents the degradation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). It is indicated for the management of type 2 diabetes mellitus in combination with metformin, a sulfonylurea or a thiazolidinedione in patients with inadequate glycaemic control following monotherapy.[3]

GLP-1 receptors have been reported to be widely expressed in the heart and vasculature of both rodents and humans, with specific localization in vascular smooth muscle, cardiomyocytes, endocardium and coronary endothelium/smooth muscle[4,5]; suggesting that GLP-1 may play an important role in the cardiovascular system.

Many studies have reported GLP-1 receptor agonists to exert wide ranging cardiovascular effects, such as modulation of heart rate, blood pressure, vascular tone and myocardial

contractility.[6, 7 ,8] Beneficial actions of these agents on cardiovascular diseases have also been reported in both experimental models and in human patients, either in the presence or absence of diabetes.[9, 10]

The majority of studies on the potential beneficial role of GLP-1 in CVD have focused on its actions in the ischemic heart and its ability to protect cardiac myocytes from ischemic damage. Several studies using various experimental models have reported that acute GLP-1 treatment exerts beneficial effects after ischemia and successful perfusion.[11, 12, 13, 5] The aim of the present study was to explore the potential protective effect of DPP-4 inhibitor on acute myocardial infarction induced experimentally in rats and possible mechanism of action.

MATERIAL AND METHODS

Experimental Protocol

1-Animal used

Adult white male albino-rats, weighting 200-250 gm were used. They were brought from Experimental Animal Breeding Farm, Helwan .All animals were housed in controlled laboratory condition at 20 -25ºC in a 12h light/dark cycle and had free access to food and water. They have acclimatized for one week and were caged (6 per cage) in fully ventilated room (at room temperature) in Pharmacology Department, Benha Faculty of Medicine. The experimental protocol met the national guiding on the proper care and use of animals in the laboratory research and an institutional animal ethics committee approved the study.

2- Experimental design

Animals were divided into the following groups.

Group (1): normal control group *(n=24).* Fed normal diet, received physiological saline in an equivalent volume of sitagliptin used.

Group (2): The myocardial infarction group (*n*=24). Rats were received physiological saline similar to the control group for 2 weeks before the induction of infarction by the ligation of the left anterior descending coronary artery (LAD).

Group (3): The sitagliptin group *(n=24)* rats received sitagliptin (5mg/kg/daily, orally) for 2 weeks prior to the ligation of LAD as described by Tsung-Ming Lee, et al.[9]

Induction of acute myocardial injury

The rats were anaesthetized with sodium pentobarbital (40 mg/kg. i.p.) throughout the surgical procedures. An endotracheal tube was inserted into the trachea to allow artificial ventilation (20 strokes/min.). Body temperature was kept constant at 37˚ C all through. Needle electrodes were inserted subcutaneously (s.c.) to monitor lead II of electrocardiograph (ECG) which was found to be the most suitable for demonstration of myocardial ischemia in rats.[14] A mid-sternal thoracotomy was performed and the heart was exposed. The left anterior descending coronary artery (LAD) was isolated from the surrounding tissues and ligated tightly by 5-0 silk ligature. The LAD ligation was done to all animals except the normal control group.

Each group was subjected to the following evaluations

Electrophysiological study in the form of S-T segment variation in ECG

ECG tracings were recorded before ligation of LAD immediately, after ligation and every 30 min. up to the end of the experiment.

Blood pressure measurements for rats

Blood pressure was measured using a BP-98A indirect (tail cuff) blood pressure meter (Softron, Tokyo, Japan) after warming the animals to 37ºC for 15 minutes. All animals were acclimated to this procedure for 3 days before measurement to minimize stress-induced variations in blood pressure. At least 3 measurements were made in every session, and the mean blood pressure was calculated as the mean of 3 representative measurements differing from each other by no more than 5mm Hg. The pulse sign should be monitored to see when the pulse signal begins to become detectable and reach the maximum pulse height. The start of pulsation is viewed on the tracing and is referenced to the pressure curve signal at that point, this reading is analogous to systolic blood pressure, while, the mean blood pressure measured at stability of pulsation and referenced to the pressure curve.[15] Rats of systolic blood pressure of 140 mmHg or more were considered hypertensive.[15]

Blood and tissue sampling

Measurement of CPK serum level

Trunk blood was collected from abdominal aorta to determine the serum levels of CPK-enzymes. The blood samples (2ml each) were allowed to clot at room temperature, centrifuged at 3000 rotation/minute and the sera were separated. Samples were stored at -20 Ć in dark containers. Measurement of cardiac lipid peroxidation (malondialdehyde), glutathione peroxidase and catalase content. To determine the MDA, glutathione peroxidase,

catalase levels, cardiac tissue samples were homogenized in ice cold 150mm KCl. The MDA levels (nmol MDA/mg protein) were assayed for the products of lipid peroxidation. Malondialdehyde (MDA), an end-product of peroxidation of cell membrane lipids caused by oxygen-derived free radicals, is considered a reliable marker of oxidative stress and was determined by measurment of the chromogen obtained from the reaction of malondialdehyde with 2-thiobarbituric acid, according to Draper and Hadley.[16]

In addition, catalase (CAT) activity (Umol)/mg was determined following the method described by Johansson and Borg[17] glutathione peroxidase by the method described by Vaghasiya et al.,[18]

Measurement of myocardial TNF-α content

Estimation of TNF-α by a sandwich ELISA with (MEDGENIX TNF-α EASIA Kit) according to the method described by Corti et al.,[19]

Histopathological examination of the cardiac sections

After records were successfully completed in all rat, the animals were scarified. The hearts were quickly removed and divided equally into two longitudinal sections. One of these parts was placed in formaldehyde solution for a routine histopathological examination by the light microscopy. The other half of the cardiac tissue Was homogenized in ice cold 150mm KCl to measure thiobarbituric acid reactive substances (TBARS) a lipid perioxidation product, catalase level, glutathione peroxidase and TNF-α levels.

Statistical Analysis: Data are presented as mean± SEM. Multiple comparisons were performed using one-way analysis of variance (ANOVA) and means of every two different groups were detected with Student's t-test. The 0.05 level of probability was used as the criterion for significance.

RESULTS

1- Effect of sitagliptin on ECG: Injury current in the form of highly peaked T wave were observed in the ECG tracing (Figure 1B) of animals after 4 hrs. of LAD occlusion compared to the normal control, non - LAD occluded rats (Figure 1A). However, the pretreatment of the animals with sitagliptin before LAD occlusion returned back ECG tracing to normal (Figure 1C).

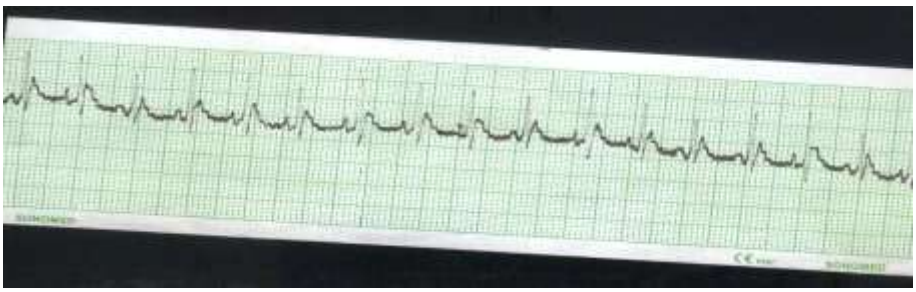
2- Effect of sitagliptin on blood pressure: nontreated myocardial infarction group showed insignificant increase in blood pressure compared with control group. Pretreatment with

sitagliptin resulted in insignificant decrease of blood pressure in compared with non-treated group (Figure 2, 3B).

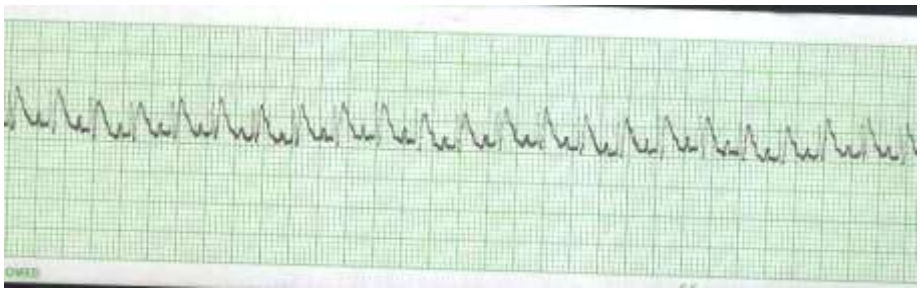
3- Effect of sitagliptin on serum CPK: The normal control rats has shown a serum CPK level of about 240 IU/L. This value was highly increased (992 IU/L) in the infracted non treated animals. Pre-treatment of the animals with sitagliptin was able to return CPK value of the infracted animals to normal without significant ($p>0.05$) changes from the control level (figure 3A).

4- Effect of sitagliptin on cardiac muscle content of MDA, catalases, glutathione peroxidase and TNF-α: MAD and TNF-α in the cardiac muscle of non-treated infracted animals were significantly higher than normal control group. On the other hand, catalase, glutathione peroxidase levels were decreased in cardiac muscle. Pretreatment of the animals with sitagliptin significantly decreased MDA and TNF-α to normal value (table 1). While, increase catalase level and glutathione peroxidase level (table 2).

5- **Effect of sitagliptin on cardiac histopathological changes:** The histopathological findings also supported the previous biochemical observations and indicated the presence of a severe form of MI in rats, which characterized by endothelial cell loss, fibrosis and myocyte hypertrophy. Pretreatment with sitagliptin significantly increased endothelial cell density, reduced myocyte hypertrophy and collagen 1 (figure 4A, B, C).



A)



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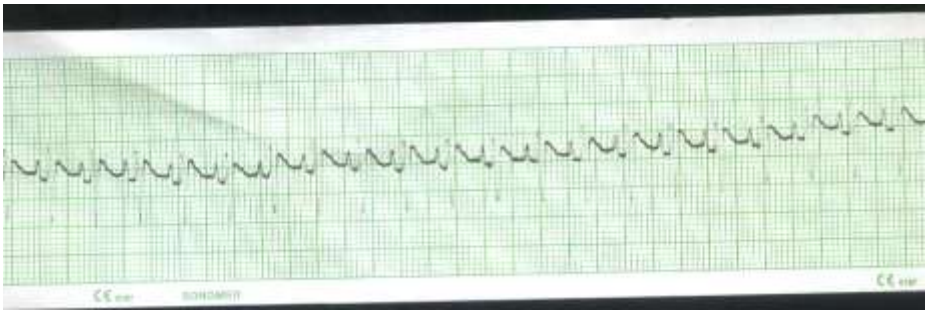
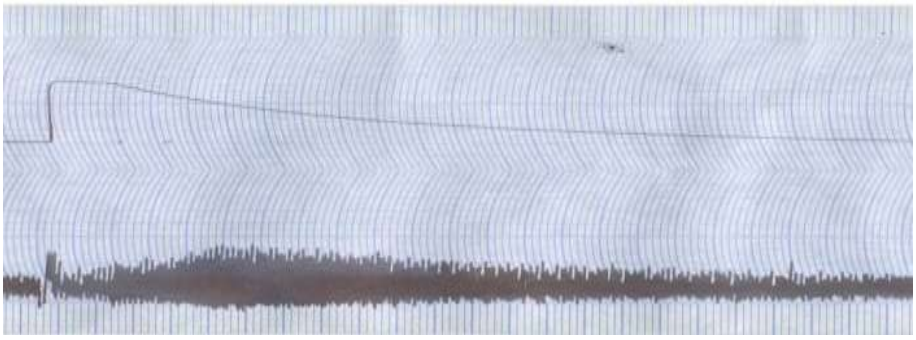
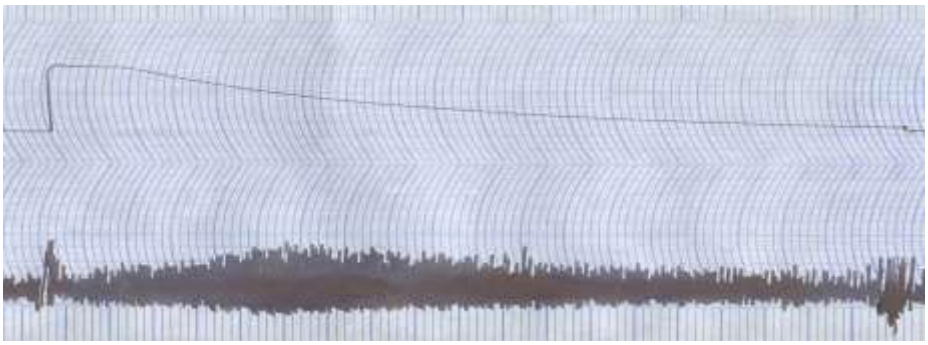


C)

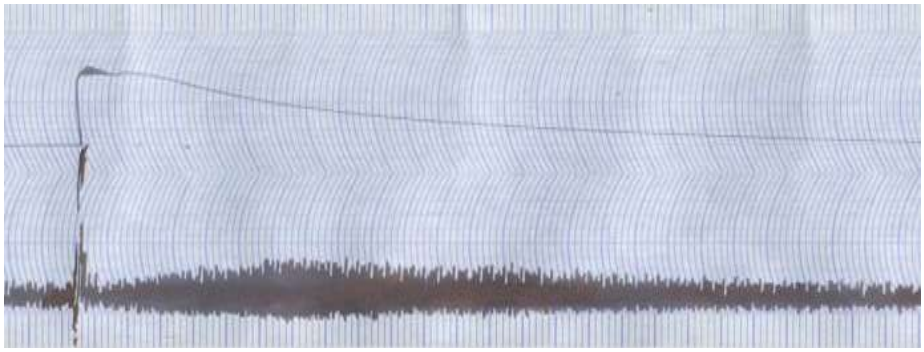
Figure (1): ECG traces showing T-wave changes and heart rate (HR) in (A) control, (B) MI and (C) sitagliptin pretreated group.



A-Blood pressure chart in control group

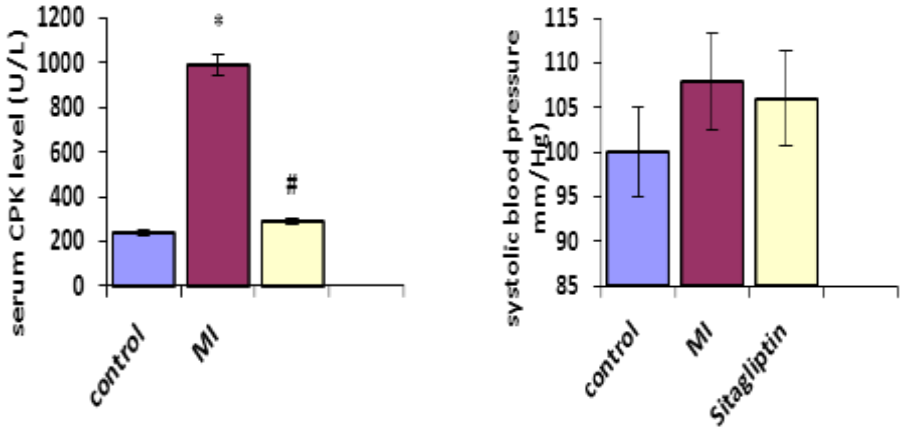


b) Blood pressure chart in myocardiac infarction non treated group.



c- Blood pressure chart in sitagliptin pretreated rats with myocardial infarction.

Figure (2): Effects of sitagliptin (5mg/kg/day, orally) pretreated rats with myocardial infarction on blood pressure showing modest decrease of blood pressure.



(A) **(B)**

Figure 1: Effect of sitagliptin pretretatment on serum CPK enzyme level (IU/L) and systolic blood pressure (mm/Hg) in myocardial infarction (MI) in rats in compare with normal control.

*: significant difference versus control group.

#: significant difference versus non sitagliptin pretreated myocardial infarction (MI) group.

Table (1): Effect of sitagliptin on cardiac malondialdehyde (umol/mg) and TNF-α (pg/ml) levels in myocardial infarction model -induced experimentally in rats.

Groups	Malondialdehyde (nmol/mg protein)	TNF-α (pg/ml)
Control	20.6±1.3	53.3± 2.5
MI nontreated	35.4±0.7*	100.5±8.1*
Sitagliptin	29.6±1.9#	75.8±5.2#

Results are mean ± SEM *, #: P<0.05

*: compared to normal control group.

#: compared to the non sitagliptin pretreated group.

Table (2): effect of sitagliptin on cardiac glutathione peroxidase (nmol/mg protein) and catalase (nmol/mg protein) levels in myocardial infarction model-induced experimentally in rats:

Groups	Glutathione Peroxidase (nmol/mg protein)	Catalase (umol/mg protein)
Control	41.5±3.2	25±1.3
MI	25.7±1.2*	9.4±0.7*
Sitagliptin	33.7±0.9#	18.3±0.1

Results are mean ± SEM ***, #: P<0.05**

*: compared to normal control group.

[#]: compared to the non sitagliptin pretreated group

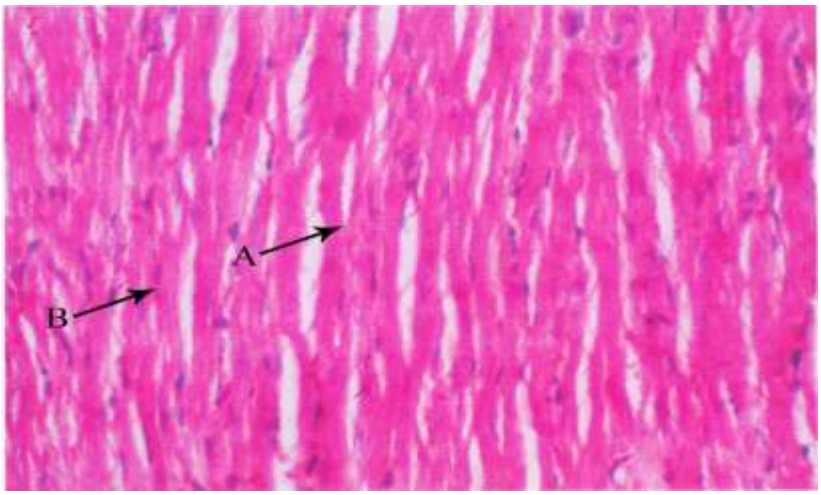


Figure (4A): A photomicrograph of a cut section in the heart of a control rat (group I) Showing interlacing (a) bundles of cardiomyocytes with (b) spindle shaped nucleus with abundant eosinophilic cytoplasm. (H&Ex40).

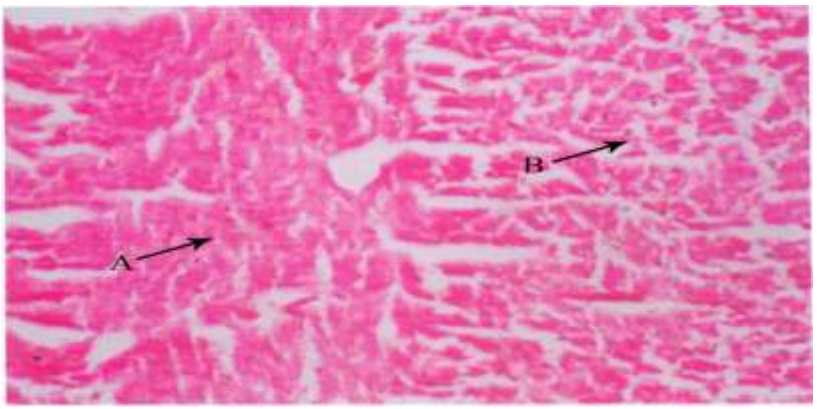


Figure (4B): A photomicrograph of a cut section in the heart of an infarcted rat (group II) Showing (a) ghosts of cardiomyocytes with cellular details. (b) Nuclei showing pyknotic changes. (H&Ex40).

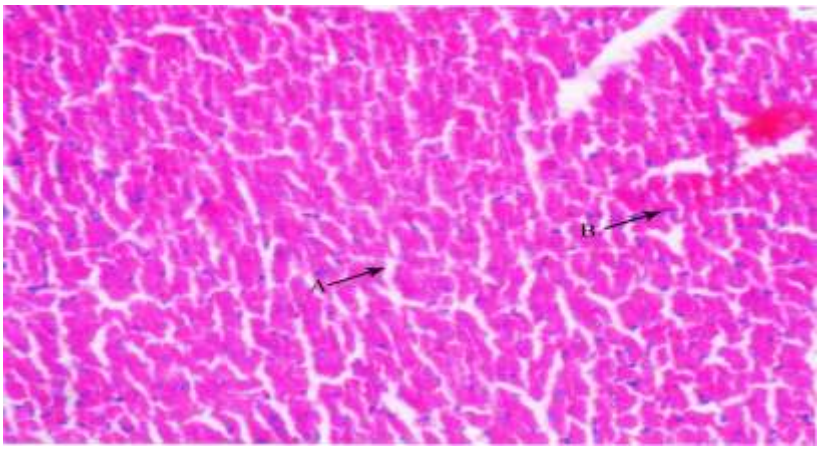


Figure (4C): A photomicrograph of a cut section in the heart of a sitagliptin pretreated rat (group VI) Showing reversal of infarction changes with (a) interlacing bundles of cardiomyocytes with (b) spindle shaped nucleus and abundant eosinophilic cytoplasm. (H&Ex40).

DISCUSSION

The present study aimed to explore the cardioprotective effect of sitagliptin against LAD ligation -induced myocardial injury, a clinically relevant animal model measuring the markers of oxidative stress, inflammation, and myocyte injury. Furthermore, to support our findings, we also examined the effects of sitagliptin on histopathological changes in the myocardium.

In the present study, induction of acute myocardial infarction significantly increased serum CPK levels. Serum CPK level have been traditionally used as important diagnostic marker enzyme. The increased level of this enzyme is indicative of severity of cell necrosis and myocardial injury. These observations are in line with previous studies which revealed that serum CPK, LDH are early and late diagnostic marker of MI.[20, 21] Pretreatment with sitagliptin significantly reduced the serum level of CPK concomitant to the histopathological preservation and inhibition of lipid peroxidation which could be reasonable to correlate with the reduced leakage of myocardial enzymes in serum.

Several studies including ours have demonstrated that MI produce marked increase in heart rate and ST-segment elevation.[22, 23, 24] Pretreatment with sitagliptin showed significant decrease in ST- segment elevation and heart rate. In accordance with this work, the results of Lee et al., [9] indicated that sitagliptin alleviated hemodynamic disturbance associated with MI by attenuating sympathetic innervation via modulating reactive oxygen species and interstitial adenosine in infarct rats.

In the present work, beside elevation of serum enzymes, cardiac content of pro-inflammatory cytokines (TNF-α) was significantly increased after MI. It is well known that the extent of MI correlates with the levels of inflammatory mediators (TNF-α) and free radical.[25, 26] Similarly, the current study confirms and extends previous findings[27, 28] that demonstrated the LAD ligation induced MI is correlates with the levels of inflammatory mediators and free radicals.[29, 30] Studies suggest that proinflammatory cytokines act as pleiotropic polypeptides that are independently associated with inflammation and oxidative stress and release of these

cytokines leads to myocardial injury through several mechanisms.[31] Following pretreatment with sitagliptin, significant reduction in the level of proinflammatory cytokines is clearly suggestive of its anti-inflammatory effect in ischemic heart. In several studies, sitagliptin has been shown to be effective in improving oxidant-antioxidant balance and reduced the levels of proinflammatory mediators and modulates several molecular pathways mediated by TNF-α.[9, 32]

Furthermore, cardiac level of MDA was remarkable increase with significantly decreased in cardiac catalase enzyme after induction of MI. In consistent with our reports, Verges et al.,[10] demonstrated that cardiac MDA level was significantly elevated at 24hrs and peaked at 48hrs after acute myocardial infarction. Lipid peroxidation is an important marker in MI and accumulation of oxidants makes the cell membranes more susceptible to oxidative injury and formation of MDA that reflects the damage of the myocardial cell contents. The decrease in MDA level following pretreatment with sitagliptin can be related to the increased activities of antioxidant status in myocardium. The antioxidant activity could be explained by a direct effect of sitagliptin on the cardiovascular system by the positive impact on heart redox status and antiapoptotic effect of the incretin modulators. Our results agree with those performed by others, which have been suggesting an antioxidant and anti-inflammatory effect of incretin modulators, due to attenuation of the deleterious effects of AGEs-RAGE-oxidative stress axis and to protection against the cytokine-induced apoptosis and necrosis.[33, 34] Several studies have demonstrated that sitagliptin efficiently scavenge free radicals and provides defense against lipid peroxidation.[35, 32] These results are in agreement with (Timmers et al. ; Noyan-Ashraf et al. ; Xie et al.; Bose et al. [36, 37, 38, 39] who suggested that GLP-1 may exert its protective effects on the ischemic myocardium, at least partly, via beneficial actions on cardiomyocyte apoptosis, oxidative stress and endogenous antioxidant defense mechanisms.

In addition, acute MI was also confirmed in this study by inflammatory features observed microscopically. Histopathological assessments revealed that induction of MI resulted in myocardial damage characterized by significant myonecrosis, edema and infiltration of inflammatory cells which attenuated by pretreatment with sitagliptin. This in agreement with previous reports by Connelly et al.,[40]; Lorber et al.[6]

CONCLUSIONS

The present study focuses on the effect of sitagliptin on experimental MI and possible anti-inflammatory and anti-oxidant effects. Results demonstrate that sitagliptin exerts

cardioprotective effect by mitigating oxidative stress, augmenting endogenous antioxidants, and maintaining structural integrity. The results of the present study indicate that sitagliptin may serve as an excellent agent alone or as adjuvant to prevent the onset and progression of myocardial injury.

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