Effect of saxagliptin and vardenafil on cardiac dysfunction and atrial natriuretic peptide gene expression in chronic isoproterenol - treated rats

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Abstract The purpose of this study was to clarify the potential cardioprotective effect of saxagliptin and vardenafil on isoproterenol-induced cardiac dysfunction. Rats were injected subcutaneously with isoproterenolas follows: doses of 30, 20, and 10 mg/kg were given on days 1, 2, and 3, respectively, followed by 5 mg/kg on days 4 to day 15. Rats received vehicle, saxagliptin (10 mg/kg) orvardenafil (10mg/kg) orally with isoproterenol to study their effects on electrocardiogram data, cardiac contractility, atrial natriuretic peptide and tumor necrosis factor α gene expression as well as histopathological analysis of cardiac tissue. Results showed that isoproterenol induced ECG changes were restored to normal by both drugs. Repeated administration of isoproterenol significantly decreased cardiac contractility by 63.62% that was increased by 53.9% with saxagliptin and by 125% with vardenafil treatment. Both treatments significantly decreased ANP and TNFα gene expression that was increased by isoproterenol injection. Histopathological examination revealed that pretreatment with either drugs led to improvement of isoproterenol- induced inflammation and vaculation and ameliorated myocardial fibrosis. In conclusion, results implicate that both saxagliptin and vardenafil may prevent isoproterenol induced cardiac dysfunction and further experimental studies are required to elucidate their potential cardioprotective mechanisms and assess their efficacy in treatment of heart failure.

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1. Introduction

Cardiac hypertrophy is a term that signifies an increased workload and is characterized with an increase in cardiac mass in response to applied stimuli. It is an important compensatory mechanism of the heart that occurs in response to different pathological stresses [1]. However, Persistent hypertrophy is ultimately associated with cardiac dysfunction [2]. The sympathoad renal systems together with hemodynamic alternations participate in the development of myocardial hypertrophy and heart failure [3]. Isoproterenol induced cardiac dysfunction has been shown to be an ideal animal model of heart failure and serves as a well-accepted standardized model to evaluate several cardiac dysfunctions [4]**.**

Atrial natriuretic peptide (ANP) is a hormone, produced mainly by cardiomyocytes, with a major role in cardiovascular homeostatic mechanisms such as natriuresis and vasodilation, which serve to regulate blood pressure [5]. The development of cardiac hypertrophy and congestive heart failure is associated with the increased expression of several fetal genes such as ANP and BNP (brain natriuretic peptide) and serum ANP levels are increased in patients with heart failure [6]**.**

Tumor necrosis factor alpha (TNF α) is a cytokine involved in systemic inflammation and it has an important role in cardiac remodeling. TNF alpha activates metalloproteinases that would be expected to activate extracellular matrix remodeling and provokes a hypertrophic growth response [7].

Dipeptidyl peptidase 4 (DPP4) inhibitors increase blood level of glucagon like peptide-1(GLP-1) and extend GLP-1 duration of action. It was reported that GLP-1 improves energy metabolism and has a protective effect on cardiomyocytes [8], and the protective role of DPP4 inhibitors in cardiovascular diseases as hypertension and myocardial infarction has been evaluated independently of their glucose-lowering action [9].

Although the cardiovascular effect of DPP4 inhibition has been substantially studied, the exact role of DPP4 in cardiovascular disease remains elusive. Recently, there is growing evidence questioning the cardioprotective effect of DPP4 inhibitors as reported from some clinical trials. Scirica et al. [10] reported an increased hospital admission rate for heart failure in saxagliptin-treated patients compared with placebo in SAVOR-TIMI 53 (The Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with

Diabetes Mellitus) trial raising the question whether DPP4 inhibitors increase the risk for heart failure.

Phosphodiesterases (PDEs) are metallo hydrolases that catalyze the breakdown of cAMP or cGMP into the inactive 5′-AMP or GMP, thus augmenting the duration and intensity of their intracellular response. phosphodiestrase -5 (PDE5) is up-regulated 2- to 6-fold in experimental mice and human heart disease potentially increasing its effect and pharmacologic impact from its subsequent inhibition [11,12].

Salloum et al. [13] demonstrated the potential cardioprotective effects of inhibitors of PDE5 especially in experimental models of congestive heart failure and left ventricular hypertrophy. Kukreja et al. [14] reported that blocking PDE-5 suppresses both chamber and myocyte hypertrophy, and improves *in vivo* heart function in mice exposed to chronic transverse aortic constriction suggesting that PDE-5 inhibition is a promising therapeutic in patients with advanced heart failure.

The present study aimed to investigate the potential protective effect of saxagliptin (a potent DPP4 inhibitor) and vardenafil (PDE-5 inhibitor) on cardiac dysfunction and atrial natriuretic peptide gene expression in chronic isoproterenol - treated rats.

2. Material and Methods

Drugs

1-Isoproterenol: It was supplied as a powder from Sigma-Aldrich (St. Louis, MO, USA). The powder is white to off- white. It was dissolved in saline and administered subcutaneously.

2- Saxagliptin: It was supplied as a powder from Sigma-Aldrich (St. Louis, MO, USA). The powder is white in color. It was dissolved in distilled water and administered orally in a dose of (10 mg /kg) half an hour before isoproterenol administration.

3- Vardenafil: It was supplied as a powder from Sigma-Aldrich (St. Louis, MO, USA). The powder is crystalline white. It was dissolved in saline and administered subcutaneously in a dose of (10 mg /kg) half an hour before isoproterenol administration.

Animals

Adult male albino rats weighing 250-300 gm were purchased from the National Institute for Research, El Doki, Cairo, Egypt. They were housed in plastic cages in a room maintained at constant temperature (21±2 C) with alternating 12 h light/dark cycle. Experiments were carried out between 8:00 am and 3:00 pm. All animal treatments adhered strictly to institutional and international ethical guidelines concerning the care and the use of laboratory animals and the experimental protocol was approved by Fayoum University Faculty of medicine Review Committee for the use of animal subjects.

Experimental procedures

A total of 32 male albino rats were divided into 4 main groups (8 rats each) and were treated for 15 days according to the following protocol:

Group I: normal control group: normal animals received saline by subcutaneous injection (s.c.).

Group II: rats received isoproterenol (s.c.) for 15 days as follows: doses of 30, 20, and 10 mg/kg were given on days 1, 2, and 3, respectively, followed by 5 mg/kg on days 4 to day 15.

Group III: rats received saxagliptin (10mg/kg) orally half an hour before isoproterenol administration.

Group IV: rats received vardenafil (10 mg/kg) orally half an hour before isoproterenol administration.

At the end of 15 days, animals were fasted for 12 hours and the following parameters were investigated:

Electrocardiogram (ECG)

Electrocardiograms were recorded for all rats under light ether anesthesia in the prone position by KENZ (ECG 106) made by NAGOYA, JAPAN. Electrodes consisted of 26-gauge needles placed subcutaneously for 1 cm. Standard limb leads were constructed from electrodes placed at the right and left forepaws and the tail [15] to measure heart rate (HR), QT interval and R wave amplitude.

Cardiac Contractility

Rats were killed and the heart, with at least 1 cm of aorta attached, was removed as quickly as possible and placed in a dish of Ringer– Locke solution (1litre of Ringer-Locke solution contains: 9g NaCl, 4.2 mL KCl (10 percent), 1g glucose, 0.5g NaHCO3, 1,08mL CaCl2 (25 percent), pure O2) at room temperature. The preparation was squeezed several times when first placed in the solution, so as to remove as much blood as possible. The aorta was located and dissected free and all other vessels connected to the heart were trimmed away. The aorta was cut just below the point where it divides and the heart was transferred to the HARVARD apparatus where the aorta was tied onto the glass cannula. The perfusion fluid was oxygenated Ringer–Locke solution. Threads were attached to the ventricle by a hook and to the auricle by a small spring clip; these threads were connected to lever to record cardiac contractility on the Kymograph [16].

Detection of cardiac ANP and TNFα gene expression by quantitative real time polymerase chain reaction (q RT – PCR)

Total RNA was extracted from heart tissue using Qiagene cells/tissue extraction kit (Qiagene, USA). Total RNA was reverse-transcribed using high capacity cDNA reverse transcription kit (#K1621, Fermentas, USA). A quantitative real-time RT-PCR was performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). The PCR primers were as follows:

* ANPprimer 1	5′GATGGATTTCAAGAACCTGCTAGAC3′
* TNFα primer 2	5′-TACAGACATTCACAGCCACC-3′

Histopathology

Animals were sacrificed and hearts were excised and preserved for 24 hours in 10% neutral buffered formalin solution. After fixation, Tissue sections from heart were subjected for routine tissue processing by [17]:

1. Dehydration which was done with a series of alcohols, 70% to 95% to 100%.
2. Clearing was done in xylol for 15 Min.
3. Embedding was performed in paraffin wax 1 hour.
4. Three serial sections sets of 5 microns thickness were obtained from the ventricle and stained with Hematoxylin and Eosin and massontrichrome for light microscopic examination.

Statistical analysis

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc, USA). For quantitative data, mean and standard deviation were calculated. ANOVA (Analysis of variance) was used to test the difference about mean values of measured parameters among groups, multiple comparison between pairs of groups were performed using LSD test (Post hoc test). For interpretation of results of tests of significance, significance was adopted at $P \leq 0.05$.

3. Results

Electrocardiogram data

Repeated Subcutaneous injection of isoproterenol for 15 days resulted in significant ($P<0.05$) increase in heart rate, QT and R wave amplitude by 31.5%, 51.2%, and 82% respectively. Pre-treatment of rats with saxaliptin significantly ($P<0.05$) decreased HR, QT and R wave amplitude by 26%, 36.2% and 51.3% respectively. Vardenafil treatment also significantly ($P<0.05$) decreased HR, QT and R wave amplitude by 25.1%, 34.3% and 55.6% respectively compared to isoproterenol group as illustrated in Table 1, 2, 3.

Cardiac contractility

Repeated administration of isoproterenol in rats significantly ($P<0.05$) decreased percentage of cardiac contractility by 63.62% as compared to normal control group.

Pretreatment of rats with saxagliptin significantly ($P<0.05$) increased percentage of cardiac contractility by 53.9% as compared to isoproterenol group, while pretreatment of rats with vardenafil significantly ($P<0.05$) increased percentage of cardiac contractility by 125% as compared to isoproterenol group Fig. 1.

Atrial natriuretic peptide (ANP) gene expression

Repeated administration of isoproterenol significantly ($P<0.05$) increased ANP gene expression compared to normal control group. Pretreatment with saxagliptin decreased the level of ANP gene expression significantly ($P<0.05$) by 67.2% as compared to isoproterenol group, while pretreatment with vardenafil decreased the level of ANP gene expression significantly ($P<0.05$) by 63.7% as compared to isoproterenol group Fig. 2a.

Tumor necrosis factor (TNF-α) gene expression

There was significant increase ($P<0.05$) in TNFα gene expression after repeated administration of isoproterenol which was decreased significantly in both saxagliptin and vardenafil treated groups by72.6% and 66.4% respectively Fig. 2b.

Histopathology

Table (1): Effect of saxagliptin and vardenafil on heart rate (beats/minute) in isoproterenol induced cardiac dysfunction in rats

Group	Mean ± SD	P-value
Normal	231.13 ± 3.23	
Saxagliptin	230.13 ± 2.22	0.651# **<0.0001##***
Vardenafil	230.50 ± 2.20	0.777# **<0.0001##***
Isoproterenol	303.75 ± 8.76	**<0.0001#***
Isoproterenol+Saxagliptin	225.00 ± 1.85	0.042#* **<0.0001##***
Isoproterenol+Vardenafil	227.25 ± 3.81	**0.142#** **<0.0001##***

Each value represents mean ± SD of 8 rats # compared group with normal group.
compared group with isoproterenol group. * Significant at P < 0.05.

Table (2): Effect of saxagliptin and vardenafil on QT interval (msec) in isoproterenol induced cardiac dysfunction in rats.

Group	Mean ± SD	P-value
Normal	103.13 ± 16.45	
Saxagliptin	102.50 ± 14.05	0.932# **<0.0001##***
Vardenafil	102.63 ± 15.17	**0.946#** **<0.0001##***
Isoproterenol	156.00 ± 7.58	**<0.0001#***
Isroterenol+Saxagliptin	99.50 ±17.33	0.625# **<0.0001##***
Isoproterenol+Vardenafil	102.25 ± 15.25	**0.906#** **<0.0001##***

Each value represents mean ± SD of 8 rats # compared group with normal group.
compared group with isoproterenol group. * Significant at P < 0.05.

Table (3): Effect of saxagliptin and vardenafil on R wave amplitude (mV) in isoproterenol induced cardiac dysfunction in rats.

Group	Mean ± SD	P-value
Normal	0.63 ± 0.16	
Saxagliptin	0.56 ± 0.11	0.358# **<0.0001##***
Vardenafil	0.56 ± 0.11	0.358# **<0.0001##***
Isoproterenol	1.15 ± 0.13	**<0.0001#***
Isoproterenol+saxagliptin	0.56 ± 0.16	0.401# **<0.0001##***
Isoproterenol+Vardenafil	0.51 ± 0.14	**0.136#** **<0.0001##***

Each value represents (mean ± standard deviation). # compared group with normal group.
compared group with isoproterenol group. * Significant at P < 0.05.

In isoproterenol group, photomicrograph showed scattered swollen cardiac muscle fibres with granular pale cytoplasm with perinuclearvacuolations, interstitium showed focal areas of oedema, congested capillaries and mononuclear inflammatory cellular infiltrate. By Masson trichrome there were large thick patches of subendocardial and interstitial fibrosis. Pretreatment with saxagliptin led to improvement of isoproterenol- induced histopathological changes. Cardiomyocytes were arranged in interlacing bundles with normal histological pattern. However, few pathological changes were still evident in the form of areas of lost striations, few degenerative changes in the form of cloudy swelling as well as few mononuclear inflammatory cells Fig. 3. By Masson trichrome there were thin and few patches of subendocardial fibrosis (green patches) were seen in one rats Fig. 4. Pretreatment with vardenafil markedly ameliorated the histopathological changes induced by isoproterenol. Cardiomyocytes were seen comparable to the control with spindle shaped nuclei and abundant eosinophilic cytoplasm Fig. 3. Mild edematous changes were seen. Masson trichrome stain showed no patches of fibrosis Fig. 4.

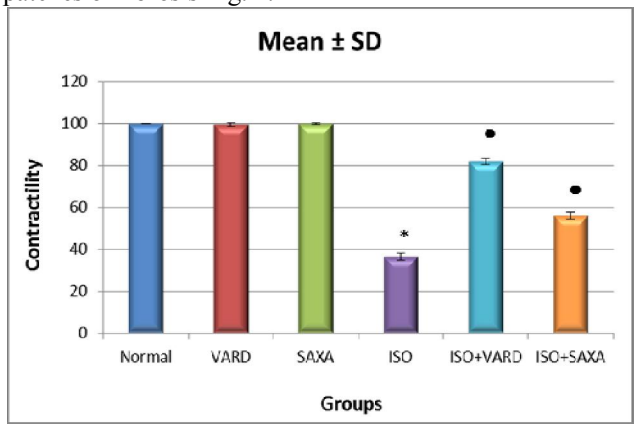
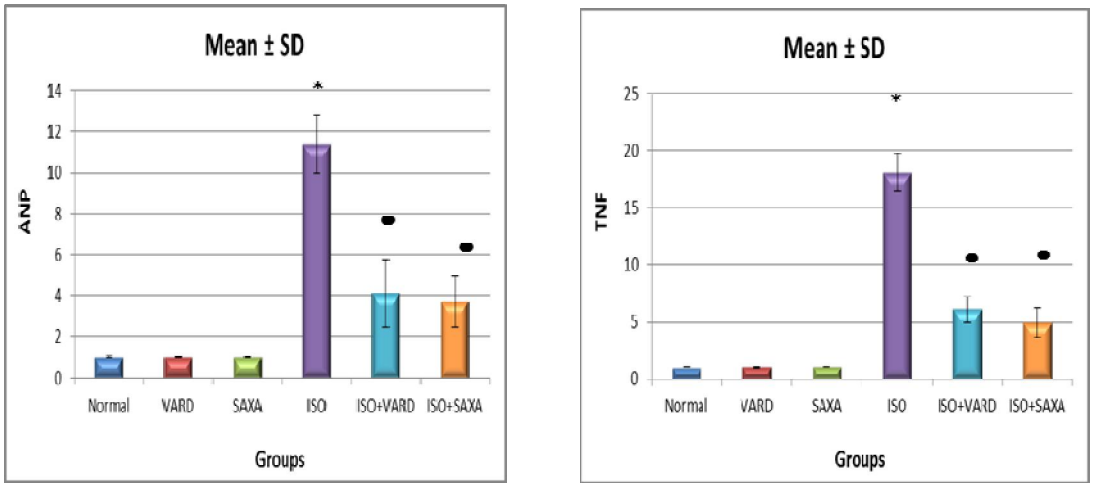


Fig (1): Effect of saxagliptin and vardenafil on cardiac contractility in isoproterenol induced cardiac dysfunction in rats. * Significant at P < 0.05 compared isoproterenol group to normal control group.● Significant at P < 0.05 compared treated groups to isoproterenol group.



(a) (b)

Fig (2): Effect of vardenafil and saxagliptin on ANP (a) and TNFα (b) gene expression in isoproterenol induced cardiac dysfunction in rats. * Significant at P < 0.05 compared isoproterenol group to normal control group.● Significant at P < 0.05 compared treated groups to isoproterenol group.

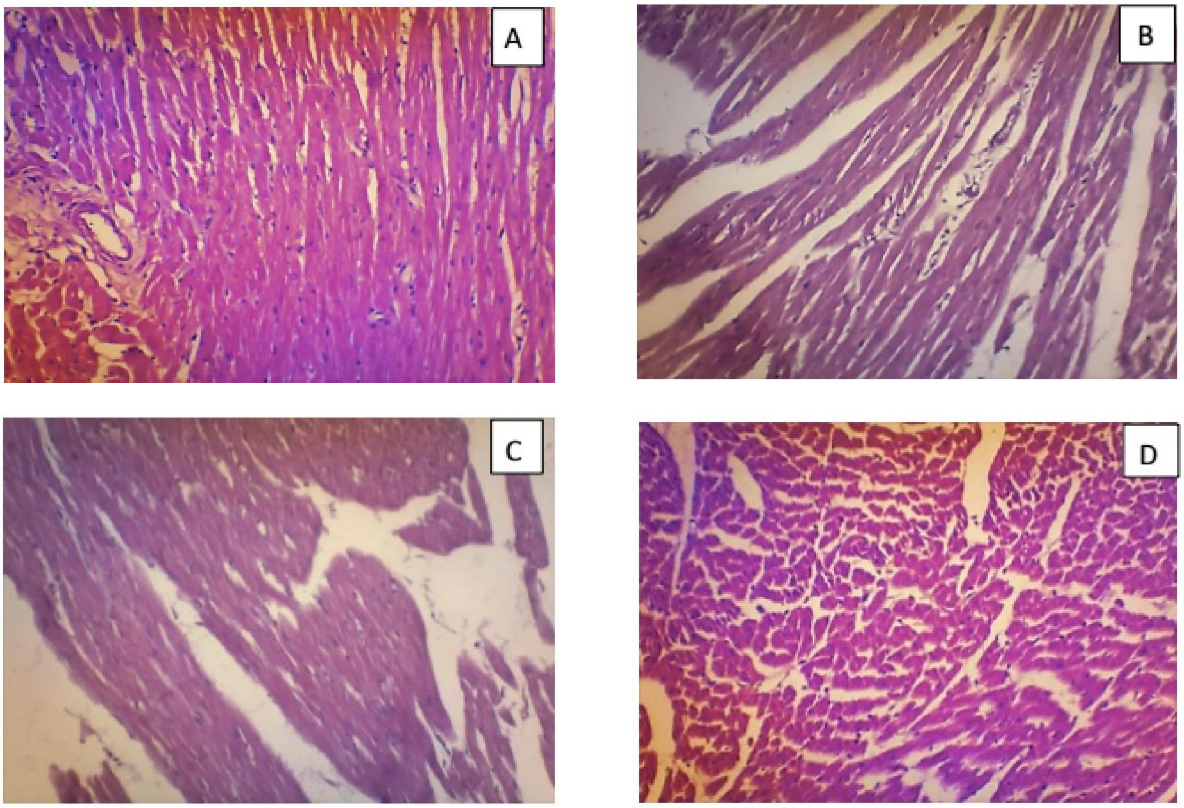


Fig (5): Effect of saxagliptin and vardenafil on isoproterenol-induced histopathologic changes in heart tissue: (A) Section in cardiac tissue of a control rat showing normal architecture of heart tissue, being composed of muscle cells, cardiomyocytes with one centrally placed nucleus; (B) Section in cardiac tissue of isoproterenol group showing focal areas of edema, congested capillaries and mononuclear inflammatory cellular infiltrates; (C) Section in cardiac tissue of saxagliptin treated group showing mild focal degenerative changes in the form of mild interstitial edema and scattered inflammatory cells; (D) Section in cardiac tissue of vardenafil treated group showing very mild focal degenerative changes in the form of cloudy swelling and mild interstitial edema (H & E 10x).

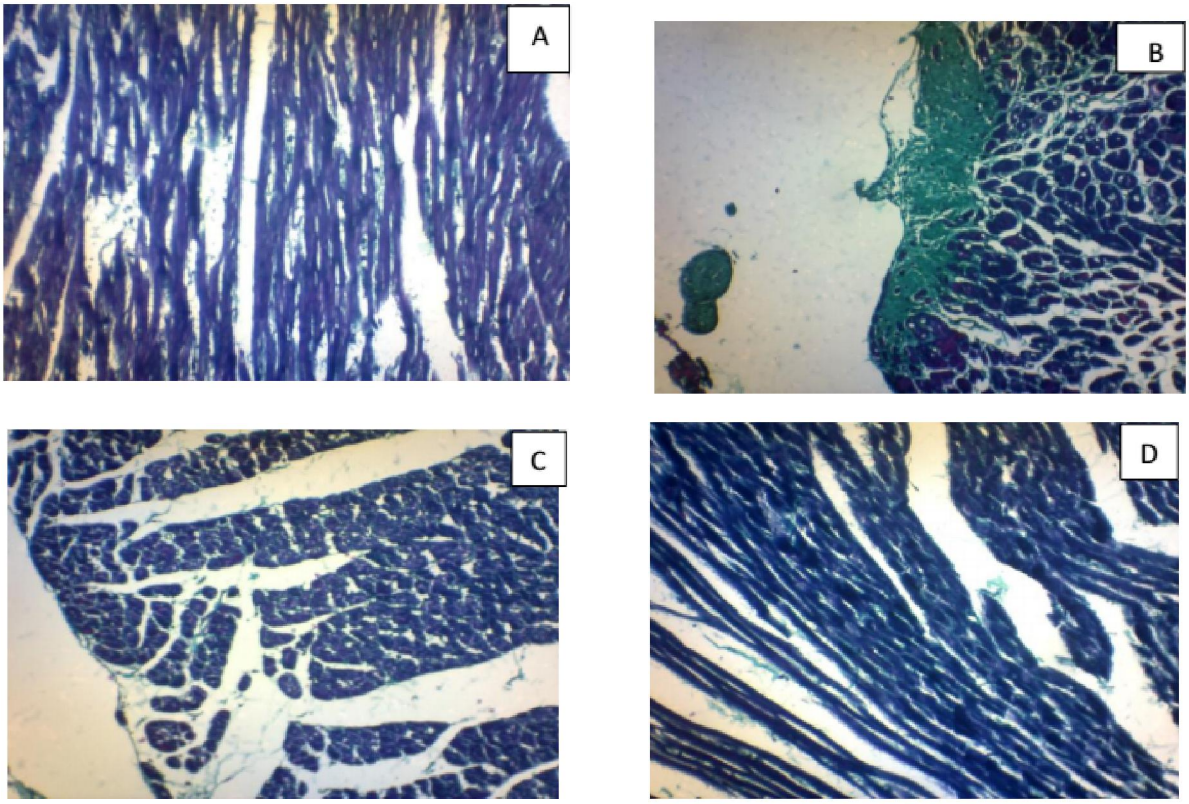


Fig (6): Effect of saxagliptin and vardenafil on isoproterenol-induced histopathologic changes in heart tissue: (A) Section in rat's heart muscle of normal control group showing no fibrosis; (B) Sections in rat's heart muscle of isoproterenol group showing large thick patches of subendocardial and interstitial fibrosis (green patches); (C) Sections in rat's heart muscle of saxagliptin treated group showing few non relevant thin patches of fibrosis; (D) Sections in rat's heart muscle of vardenafil treated group showing no fibrosis (Masson trichrome10x).

4. Discussion

The purpose of current study was to evaluate the cardioprotective properties of saxagliptin (a potent dipeptidyl peptidase 4 inhibitor) and vardenafil (a phosphodiestrase-5 inhibitor) in chronic isoproterenol induced cardiac dysfunction through their effect on ECG data, cardiac contractility, gene expression of ANP and TNF alpha.

The ECG is reliable for detecting pathological changes in isoproterenol-induced remodeling of rat heart. In the present study significant alterations of ECG patterns were observed in isoproterenol-treated rats when compared with normal rats. These changes were in the form of significant increased heart rate, QT interval and R-wave amplitude. This coincides with Kralova et al. [18] who reported that relatively short time period of administration of repeated low doses of isoproterenol induced cardiac hypertrophy producing significant changes in ECG configuration as increased amplitude of P wave, prolongation of PQ interval and QRS complex, increased amplitude of R and prolongation of QT interval. In the present study, the ECG changes induced by isoproterenol were significantly attenuated by pretreatment with saxagliptin.

In the present work repeated administration of isoproterenol in rats significantly decreased cardiac contractility by 63.62% as shown by Langendorff apparatus as compared to normal control group. Pretreatment with saxagliptin significantly increased cardiac contractility by 53.9% as compared to isoproterenol group.

As regards gene expression study, the present study revealed that pretreatment with saxagliptin decreased the elevated level of ANP and TNFα gene expression as compared to isoproterenol group. Moreover, histopathological study of the present work showed that pretreatment with saxagliptin prevented cardiomyocyte inflammation and fibrosis induced by isoproterenol.

Orally administered dipeptidyl peptidase-4 (DPP-4) inhibitors have emerged as a new class of antidiabetic agents owing to their ability to extend the biological effects of incretin hormones namely

glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [19]. Expression of GLP-1 receptors has been detected in the vasculature and both DPP-4 and specific GLP-1 receptors are expressed in the rodent myocardium [20]. There is a growing body of evidence that incretin-based drugs like exenatide or dipeptidyl peptidase inhibitors that augment endogenous GLP-1 have a cardio protective effect. Sokos et al. [21] reported that GLP-1 administered by continuous subcutaneous infusion has been shown to increase myocardial glucose delivery and improve left ventricular function in patients with heart failure. Moreover, Vyas et al. [22] demonstrated that the GLP-1agonist, exenatide, improved glucose tolerance, cardiac contractility, and survival over control vehicle-treated TG9 mice (a murine model of dilated cardiomyopathy). Kubota et al. [23] demonstrated that DPP-4 activities in cardiac tissue were upregulated in experimental models of heart failure with both cardiac diastolic and systolic dysfunction suggesting that an increase in cardiac DPP-4 activity may be associated with the development of heart failure.

Ikeda et al. [24] measured the cardiac DPP-4 activity using an in situ activity staining method and detected DPP-4 staining that was localized in the endothelium of the venous capillary vessels in cardiac tissues. They found that Saxagliptin significantly inhibited cardiac DPP-4 activities in isoproterenol-treated rats. Moreover, saxagliptin treatment prevented the cardiac hypertrophy and fibrosis induced by the continuous infusion of isoproterenol and this effect was accompanied by the suppressed expression of genes related to hypertrophy, such as ANP, interleukin-6 (IL-6) and insulin-like growth factor 1 (IGF-1) and pro-fibrotic factors, such as collagen I and collagen III. They concluded that saxagliptin exerts its cardioprotective effect by the inhibition of endothelial DPP-4 activity in cardiac tissue, independently of its glycemic action. Miyoshi et al. [25] studied another DPP-4 inhibitor (Vildagliptin) on isoproterenol induced cardiac hypertrophy in rats. Cardiac hypertrophy was introduced by subcutaneous isoproterenol (2.4 mg/kg/day) for 7 days. He reported that, LV hypertrophy was significantly decreased in the isoproterenol- vildagliptin group compared with the isoproterenol group. Vildagliptin also lowered the elevated left ventricular end-diastolic pressure observed in the isoproterenol group. Moreover, Histological analysis showed that vildagliptin attenuated the increased cardiomyocyte hypertrophy and perivascular fibrosis. Furthermore, Quantitative PCR showed attenuation of increased mRNA expression of TNFα in the isoproterenol- vildagliptin group.

Kukreja et al. [14] reported that blocking PDE-5 suppresses both chamber and myocyte hypertrophy, and improves *in vivo* heart function suggesting that PDE-5 inhibition is a promising therapeutic target in patients with advanced heart failure.

Results of the present study showed that pretreatment of rats with vardenafil significantly decreased heart rate, QT interval, R wave amplitude and increased cardiac contractility as compared to isoproterenol group. Moreover, histopathological study of the present work showed that pretreatment with vardenafil markedly ameliorated the histopathological changes induced by isoproterenol. Cardiomyocytes were seen comparable to the control with spindle shaped nuclei and abundant eosinophilic cytoplasm and Masson trichrome stain revealed no fibrosis.

Hassan and Ketat [26] reported that prior administration of a PDE-5 inhibitor before isoproterenol for 10 days improved the survival of rats and protected the heart so the myocardium did not show hypertrophy or cell injury. They suggested that PDE-5 inhibitors exert their cardio protective effect through increasing cardiac cGMP level which acts as a post-receptor negative regulator of cardiac sympathetic responsiveness. Salloum et al. [27] reported that PDE-5 inhibitors have a positive inotropic effect in rats with cardiac hypertrophy especially with high doses which could be through different mechanism other than PDE-5 inhibition. This result also matches Lindman et al. [28] who stated that PDE-5 is expressed in the hypertrophic ventricle and that PDE-5 inhibition has a direct inotropic effect in rats with right ventricular hypertrophy which could be through direct effect.

In the present study Pretreatment with vardenafil before isoproterenol significantly decreased the elevated level of ANP and TNFα gene expression as compared to isoproterenol group. This is in agreement with the study of Lubamba et al. [29] who stated that treatment with PDE-5 inhibitors strongly suppressed the expression of the hypertrophy marker genes such as ANP and that vardenafil attenuated neutrophil cell infiltration and inflammatory cytokines such as TNFα which could be attributed to the consequent increase in intracellular cGMP.

Conclusion

The results of the present study add an idea about the cardioprotective effect of saxagliptin and vardenafil by ameliorating isoproterenol -induced electrocardiographic, cardiac contractility, and histopathological alternations and by decreasing the expression of genes related to cardiac dysfunction as ANP and TNFα.

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References

1 Akazawa H, Kumuro I. Roles of cardiac transcription factors in cardiac hypertrophy. Circ Res 2003; 92: 1079-1088.
2 Armoundas AA, Wu R, Juang G, Marban E, Tomaselli GF. Electrical and structural remodeling of the failing ventricle. Pharmacol Ther 2001; 92: 213-230.
3 Šimko F. Left ventricular hypertrophy regression as a process with variable biological implications. Can J Cardiol 1996; 12: 507-513.
4 Upaganlawar A, Gandhi H, Balaraman R. Isoproterenol induced myocardial infarction: protective role of natural products. Jpharmacol and toxicol 2011; 6(1): 1-17.
5 De Vitoa P, Incerpib S, Pedersena J Z., Lulya P. Atrial natriuretic peptide and oxidative stress. Peptides 2010; 31:1412–1419.
6 Federico C. natriuretic peptide system and cardiovascular disease. Heart Views 2010; 11(1): 10–15.
7 Galeone A, Paparella D, Colucci S, Grano M, Brunetti G. The Role of TNF-α and TNF Superfamily Members in the Pathogenesis of Calcific Aortic Valvular Disease. Scient World J 2013; 88: 45-55.
8 Pacheco BP, Crajoinas RO, Couto GK, Davel AP, Lessa LM, Rossoni LV et al. Dipeptidyl peptidase IV inhibition attenuates blood pressure rising in young spontaneously hypertensive rats. J Hypertens 2011; 29(3): 520–528.
9 Sumita YW, Nakamura K, Miura D, Shimizu J, Hashimoto K, Kataoka N et al. Increased Passive Stiffness of Cardiomyocytes in the Transverse Direction and Residual Actin and Myosin Cross-Bridge Formation in Hypertrophied Rat Hearts Induced by Chronic beta-Adrenergic Stimulation. Circ 2012; 77(3):741–748.
10 Scirica B M, Bhatt D L, Braunwald E, Steg P G, Davidson J, Hirshberg B et al."Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus," The New England Journal of Medicine 2013; 369(14): 1317–1326, 2013.
11 Nagendran J, Archer S L, Soliman D, Gurtu V, Moudgil R, Haromy A et al. Phosphodiesterase type 5 is highly expressed in the hypertrophied human right ventricle, and acute inhibition of phosphodiesterase type 5 improves contractility. Circulation 2007; 116,238–248.
12 Pokreisz P, Vandenwijngaert S, Bito V, Van den Bergh A, Lenaerts I, Busch C, et al. Ventricular phosphodiesterase-5 expression is increased in patients with advanced heart failure and contributes to adverse ventricular remodeling after myocardial infarction in mice. Circulation 2009;119,408–416.
13 Salloum FN, Chau VQ, Hoke NN, Abbate A, Varma A, Ockaili RA et al. Phosphodiesterase-5 inhibitor, tadalafil, protects against myocardial ischemia/reperfusion through protein-kinase g-dependent generation of hydrogen sulfide. Circ 2009; 120:31-36.
14 Kukreja RC, Salloum FN, Das A, Koka S, Ockaili RA, Xi L. Emerging new uses of phosphodiesterase-5 inhibitors in cardiovascular diseases. Exp Clin Cardiol 2011; 16:30–35.
15 Grant A. O. Cardiac ion channels. Circ. Arrhyth. Electrophysiol 2009; 2: 185–194.
16 Perry, WLM. Pharmacological experiments on isolated preparations. 2nd ed. Edinburgh: Churchill Livingstone; 1970.
17 Culling, C F A. Handbook of histopathological and histochemical techniques.3rd ed. Butterworth- Heinemann: London, UK; 1974.
18 Kralova E, Mokran T, Murin J, Stankovicova T. Electrocardiography in Two Models of Isoproterenol-Induced Left Ventricular Remodeling. Physiol. Res 2008; 57: 83-89.
19 Kim D, Wang L, Beconi M, Eiermann GJ, Fisher MH, He Het al. (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6- dihydro [1,2,4] triazolo [4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. J. Med. Chem 2005; 48:141–151.
20 Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 2008; 117:2340–2350.
21 Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. J Card Fail 2006; 12:694–699.
22 Vyas A K, Yang K C, Woo D, Tzekov A, Kovacs A, Jay P Y et al. Exenatide Improves Glucose Homeostasis and Prolongs Survival in a Murine Model of Dilated Cardiomyopathy. PLoS One 2011; 6(2): e17178. doi: 10.1371/journal.pone.0017178.
23 Kubota A, Takano H, Wang H, Hasegawa H, Tadokoro H, Hirose M, et al. DPP-4 inhibition

has beneficial effects on the heart after myocardial infarction. J Mol Cell Cardiol 2016; 91:72e80.

24 Ikeda J, Kimoto N, Kitayama T, Kunori S. Cardiac DPP-4 inhibition by saxagliptin ameliorates isoproterenol-induced myocardial remodeling and cardiac diastolic dysfunction in rat. Journal of Pharmacological Sciences2016; 132(1): 65-70.

25 Miyoshi T, Nakamura K, Yoshida M, Miura D, Oe H, Akagi S et al. Effect of vildagliptin, a dipeptidyl peptidase 4inhibitor, on cardiac hypertrophy induced by chronic beta-adrenergic stimulation in rats. Card Diab 2014; 13:43. doi: 10.1186/1475-2840-13-43.

26 Hassan M, Ketat A. Sildenafil citrate increases myocardial cGMP content in rat heart, decreases its hypertrophic response to isoproterenol and decreases myocardial leak of creatine kinase and troponin T. BMC Pharmacol 2005; 5:10-19.

27 Salloum FN, Chau VQ, Hoke NN, Abbate A, Varma A, Ockaili RA et al. Phosphodiesterase-5 inhibitor, tadalafil, protects against myocardial ischemia/reperfusion through protein-kinase g-dependent generation of hydrogen sulfide. Circ 2009; 120:31-36.

28 Lindman BR, Zajarias A, Madrazo JA. Effects of phosphodiesterase type 5 inhibition on systemic and pulmonary hemodynamics and ventricular function in patients with severe symptomatic aortic stenosis. Circulation 2012; 25(19): 2353–2362.

29 Lubamba B., Huaux F, Lebacq J, Marbaix E, Dhooghe B, Panin N et al. Immunomodulatory activity of vardenafil on induced lung inflammation incystic fibrosis mice. J Cystic Fibrosis 2012; 11: 266–273.

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