Effect of GLP-1 on after Experimental Ischemic Reperfusion Injury in Rats

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Abstract: Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by L-cells of small intestine in response to nutrient ingestion. Although the major physiological function of GLP-1 appears to be in relation to glycaemic control, there is growing evidence to suggest that it may also play an important role in the cardiovascular system. GLP-1 receptors (GLP-1Rs) are expressed in the heart and vasculature of both rodents and humans, and recent studies have demonstrated that GLP-1R agonists have wide-ranging cardiovascular actions, such as modulation of heart rate, blood pressure, vascular tone and myocardial contractility. In this study the cardiac effect of native GLP-1 after experimental induction of ischemia was studied. Fifty rats were used in this study. Their weight ranged from 200-250 grams. Rats were anesthetized and the hearts were excised. The hearts were mounted on a Langendorff perfusion system and a retrograde perfusion was started within 3min of the heart excision. After 30 min of stabilization, the following groups were defined: **(1) Control group (group1a)**: consists of 10 rats, no ischemia, the flow was continuous for 2 hours. (Sham operation). In the other groups the flow was turned off for 35 min to elicit global ischemia and **reperfusion** was continues for 120min. **(2) Control group (group1b)**: consists of 10 rats, no pharmacological agents were added during the first 15min of reperfusion. **(3) DPP4 inhibitor group (group2)**: consists of 10 rats, sitagliptin 20mg/l was added during the first 15min of reperfusion. **(4) GLP-1group (group3)**: consists of 10 rats, GLP-1 (0.3nM/l) + sitagliptin 20mg/l were added during the first 15min of reperfusion. **(5) GLP-1 high dose group (group4)**: consists of 10 rats, GLP-1(10.3nM) + sitagliptin 20mg/l were added during the first 15min of reperfusion**.** The following parameters were measured at the end of stabilization period (reading1) and at the end of reperfusion (reading2) 1- Heart rate / min. 2-Left ventricular developed pressure/mmHg =left ventricular systolic-diastolic pressure. 3-Rate pressure product/mmHg/min =HR × LVDP and 4-Maximum rate of pressure rise $\Delta P/\Delta T$ /mmHg/sec. Histopathological studies of sections from the 5 studied groups were performed using Hematoxylin and Eosin.

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1. Introduction:

Glucagon-like peptide 1 (GLP-1) is a 30–amino acid gut hormone secreted from intestinal L-cells in response to nutrient ingestion. It stimulates insulin secretion, increase feeling of satiety, inhibits glucagon secretion and gastric emptying, thereby reducing postprandial glycemia[(1)]

GLP-1 is derived from posttranslational proteolysis of preproglucagon, and its peptide sequence is identical in Mouse, rat, and humans. Active isoforms of GLP-1 include GLP-1(7-36) amide and glycine -extended GLP-1(7-37)[(2)].

The half-life of intact GLP-1 is extremely short (2 min), in part due to renal clearance. GLP-1 is also rapidly metabolized to GLP-1(9-36)amide by the enzyme dipeptidyl peptidase-4 (DPP-4), which is abundantly expressed in many cell types.GLP-1(9-36) does not interact with the known GLP-1 receptor [(3)]**.**

The GLP-1 receptors are widely expressed in islet cells, kidney, lung, brain, the gastrointestinal tract, and in the heart[(4)]. Stimulation of the receptor results in arise in intracellular (cAMP) and calcium concentration, which in β cells is a signal for exocytosis of previously synthesized insulin[(5)].

GLP-1 has many protective functions on heart. In this research we study its effect in myocardial ischemia. Myocardial ischemia and infarction is one of the most important causes of death.

Myocardial infarction (MI) results in irreversible loss of Cardiomyocytes. Restoration of the antegrade coronary flow in the infarct-related coronary artery limits myocardial ischemic necrosis and is the cornerstone treatment of ST-segment elevation myocardial infarction (STEMI). However, despite adequate reperfusion, most patients still suffer irreversible myocardial cell loss, which is partly caused by the reperfusion itself. Reperfusion induces several abrupt biochemical and metabolic changes, including the generation of reactive oxygen species leading to oxidative stress, intracellular calcium overload, the rapid restoration of physiologic pH, and inflammation[(6)]. These changes eventually interact to

accelerat myocardial apoptosis through the opening of the mitochondrial permeability transition pores.

Aim of work

To study effect of GLP-1 on heart after experimental ischemia and reperfusion& determination of infarction size

2. Experimental Design:

1- Heart isolation

The rats were anesthetized with a mixture of Midazolam /sterile water (1:2) by s.c. injection (0.2 ml/kg body weight), and when sedated, heparin (1000 IE/kg) was injected via the femoral vein. During the operative procedure, the rats were ventilated with mixture of 35% O_2, 65% N_2 via a tracheotomy, to ensure arterial pH 7.35–7.45. Following thoracotomy, the heart was excised, and placed in a Petri dish containing ice-cold (<4◦C) perfusion buffer. Lung and fat tissue were removed. **(Alvilde *et al.*, 2009*)***

2- Ischemia reperfusion Protocol

The heart was mounted on a Langendorff perfusion system and a retrograde perfusion was started within 3min of the heart excision. The perfusion buffer was modified Krebs–Henseleit solution maintained at 37◦C. To determine left ventricular pressure, left auricle was excised and size 7-balloon placed inside the left ventricle, and balloon volume adjusted to a diastolic pressure of 4–10mmHg. Hearts were then placed in water jacketed heart chamber (Radnotti, Harvard apparatus, USA) maintained at 37 ºC and allowed to stabilize for 30min. Constant pressure perfusion was employed, with the pressure slowly adjusted to 80mmHg at the beginning of the stabilization period. The perfusion buffer was modified Krebs–Henseleit solution, consisting of (concentrations in mM): NaCl 118.5, KCl 4.7, NaHCO3 25.0, $MgSO_4$ 1.2, $CaCl_2$ 1.2, glucose 11.1 equilibrated to pH 7.4 with a gas mixture of 5% CO_2/ 95% O_2, and maintained at 37◦C. Successful retrograde coronary perfusion was ascertained by collecting coronary effluent, a volume of 20ml is considered an indication for successful perfusion[7]**.**

Hearts were left to beat spontaneously and beating was checked throughout the experiment.

Perfusion and treatment protocols in groups:

Following 30 min stabilization, the following groups were defined:

1. **Control group (group1a)**: consists of 10 rats, no ischemia, the flow was continued for 2 hours. (Sham operation)

In the other groups the flow was turned off for 35 min to elicit global **ischemia** and **reperfusion** was continues for 120min.

2. **Control group (group1b)**: consists of 10 rats, no pharmacological agents were added during the first 15min of reperfusion.
3. **DPP4 inhibitor group (group2)**: consists of 10 rats, sitagliptin 20mg/l was added during the first 15min of reperfusion.
4. **GLP-1group (group3)**: consists of 10 rats, GLP-1 (0.3nM/l) + sitagliptin 20mg/l were added during the first 15min of reperfusion.
5. **GLP-1 high dose group (group4)**: consists of 10 rats, GLP-1(10.3nM/l) + sitagliptin 20mg/l were added during the first 15min of reperfusion [8]**.**

To monitor mechanical performance of the hearts, the input signal from balloon pressure transducer was recorded. Digital analysis of the wave was then performed and displayed by an electronic polygraph.[9]

Baseline measurements were then recorded at the end of stabilization period **(reading 1).**

Measured parameters:

Left ventricular function was assessed by

1- Left ventricular developed pressure (LVDP) defined as peak systolic minus end-diastolic pressure, left ventricular end-diastolic pressure (LVEDP),

1. Maximum rate of pressure rise ΔP/ΔT max (1&2 two sensitive indices for contractility),

2- Rate pressure product RPP the product heart rate × left ventricular developed pressure (HR X LVDP) which correlates well with the cardiac work.

At the end of stabilization period ischemia was achieved by clamping the aortic cannula to achieve zero flow in groups (1b,2,3,4) .To induce myocardial infarction no flow ischemia was maintained for 35min. Hearts were then reperfused with the same KH solution and post ischemic contractile parameters were recorded at 120min. **(reading 2)**

At the end of experimental period, hearts were collected and immediately stored in 10% buffered formalin for pathological studies.

4- Determination of infarct size

Infarct size was determined and expressed as a percentage of area-at-risk (% IS/AAR) as follows:

Preparation of paraffin blocks:

Specimens were fixed immediately in 10% buffered formalin. Paraffin embedded tissues were cut into thin sections of 5µm thickness in Pathology department, Faculty of Medicine, Cairo University.

Routine Hematoxylin & Eosin staining ***(*Drury and Wallington, 1976*)***:

Statistical Analysis:

Data were coded and entered using the statistical package SPSS (V.16.).Data were summarized using: Mean and Standard deviation (SD).

Comparisons between groups were done using Analysis of Variance (ANOVA) and multiple comparisons by (Post-Hoc test) for normally

distributed quantitative variables. While quantitative variables which are not normally distributed were compared using non parametric tests (Kruskal-Wallis test). P-values less than or equal to 0.05 were considered statistically significant.

3. Results

Table (1): Comparison between groups # in heart rate, LVDP, RPP and ΔP/ΔT at end of stabilization period (reading 1)

	Group 1a	Group 1b	Group 2	Group 3	Group4	*P* value
HR/min Mean ±SD	149.6±1.3	148 ±7	148± 7	151±10	150±6	0.728†
LVDP Mean ±SD	80.1±0.99	79 ± 6	81± 6	83± 6	81±7	0.646†
RPP Mean ±SD	12000±222.4	12000±979	12000±792	12600±1211	12300±1143	0.464†
Dp/dt Mean ±SD	1322±16.2	1300±169	1340±188	1337±155	1324±145	0.897†

#ANOVA test was done. † Insignificant difference

Table (2): Comparison# between control group 1a and control group 1 at end of perfusion

	Control group 1a	Control group 1b	*P* value
HR mean± SD	149±1.3	107 ± 6	<0.01*
LVDP mean± SD	80±0.99	45 ± 3	<0.01*
RPP mean± SD	12000±222	4862 ± 482	<0.01*
DP/DT mean± SD	1322±16.2	740 ± 44	<0.01*

ANOVA test was done. * Significant difference (p<0.05)

Table (3):Heart rate, LVDP, RPP and ΔP/ΔT at end of reperfusion in different groups (reading 2).

	Group1b	Group 2	Group 3	Group 4
HR Mean± SD	107±6*	104±5•	131±8▪	131±6▫
LVDP Mean± SD	45±3*	39±3•	51±1▪	54±3▫
RPP Mean± SD	4862±482*	4167±286•	6704± 511▪	7099±481▫
DP/DT Mean± SD	740±44*	677±5•	820±35▪	839±48▫

Table (3) shows differences between groups in mean values of heart rate/min, LVDP (mmHg), RPP (mmHg/min) and ΔP/ΔT (mmHg/sec) at end of reperfusion, so inter group comparison was done.

Table (4): Inter group # comparison in heart rate/min at end of reperfusion.

Dependent variable	Group(I)	Group (J)	Mean difference (I-J)	*P* value
HR/ min 120 min	1b (control Group)	2	2.30	0.456†
		3	-24.0	<0.01*
		4	-24.40	0.000*
	2 (DPP-4 Inhibitor group)	1	-2.30	0.456†
		3	-26.30	<0.01*
		4	-26.70	0.000*
	3 (GLP- 1 low dose group)	1	24.0	<0.01*
		2	26.30	0.000*
		4	-.40	.897†
	4 (GLP-1 high dose group)	1	24.40	<0.01*
		2	26.70	0.000*
		3	.40	0.897†

post-hoc test was done * Significant difference (p<0.05) † Insignificant difference

Table (4): explains that there was no significant difference between groups 1b &2 as regard heart rate at end of reperfusion $\boldsymbol{p}$ >0.05.

There was no significant difference between groups 3&4 as regard heart rate at end of reperfusion $\boldsymbol{p}$ > 0.05.

There was significant difference between groups 1b& 3 as regard heart rate at end of reperfusion $\boldsymbol{p}$ < 0.05.

There was significant difference between groups 1b& 4 as regard heart rate at end of reperfusion $\boldsymbol{p}$ < 0.05.

There was significant difference between groups 2& 3 as regard heart rate at end of reperfusion $\boldsymbol{p}$ < 0.05.

There was significant difference between groups 2& 4 as regard heart rate at end of reperfusion $\boldsymbol{p}$ < 0.05.

Table (5): Inter group comparison# in LVDP/mmHg at end of reperfusion.

Dependent variable	Group(I)	Group (J)	Mean difference (I-J)	*P* value
LVDP 120 min	1 b (control Group)	2	5.20	0.001*
		3	-6.10	<0.01*
		4	-9.00	0.000*
	2 (DPP-4 Inhibitor group)	1	-5.20	0.001*
		3	-11.30	<0.01*
		4	-14.20	.000*
	3 (GLP-1 low dose group)	1	6.10	<0.01*
		2	11.30	0.000*
		4	-2.90	0.044†
	4 (GLP-1 high dose group)	1	9.0	0.000*
		2	14.20	<0.01*
		3	2.90	0.044†

#Post- Hoc test was done * Significant difference (p<0.05) † Insignificant difference

Table (5): explains that there was significant difference between groups 1b&2 as regard LVDP at end of reperfusion $\boldsymbol{p} < 0.05$.

There was significant difference between group 3&4 as regard LVDP at end of reperfusion $\boldsymbol{p} < 0.05$. There was significant difference between groups 1b&3 as regard LVDP at end of reperfusion $\boldsymbol{p} < 0.05$.

There was significant difference between group 1b& 4 as regard LVDP at end of reperfusion $\boldsymbol{p} < 0.05$. There was significant difference between group 2 &3 as regard LVDP at end of reperfusion $\boldsymbol{p} < 0.05$.

There was significant difference between group 2 &4 as regard LVDP at end of reperfusion $\boldsymbol{p} < 0.05$.

Table (6): Inter group Comparison #in RPP/mmHg/min at end of reperfusion

Dependent variable	Group(I)	Group (J)	Mean difference (I-J)	*p* value
RPP 120 min	1b(control group)	2	659.0	0.002*
		3	-1878.50	<0.01*
		4	-2273.20	0.000*
	2 (DPP-4 Inhibitor group)	1	-659.0	0.002*
		3	-2537.50	<0.01*
		4	-2932.20	0.000*
	3 (GLP-1 low dose group)	1	1878.50	<0.01*
		2	2537.50	0.000*
		4	-394.70	0.057†
	4 (GLP-1 high dose group)	1	2273.20	0.000*
		2	2932.20	<0.01*
		3	394.70	0.057†

#Post-Hoc test was done * Significant difference (p<0.05) † Insignificant difference

Table (6): explains that there was significant difference between groups 1b & 2 as regard RPP at end of reperfusion $\boldsymbol{p < 0.05}$.

There was **no** significant difference between group 3&4 as regard RPP at end of reperfusion $\boldsymbol{p > 0.05}$.

There was significant difference between group 1b& 3 as regard RPP at end of reperfusion $\boldsymbol{p < 0.05}$.

There was significant difference between group 1b&4 as regard RPP at end of reperfusion $\boldsymbol{p < 0.05}$. There was significant difference between group 2&3 as regard RPP at end of reperfusion $\boldsymbol{p < 0.05}$.

There was significant difference between group 2&4 as regard RPP at end of reperfusion $\boldsymbol{p < 0.05}$.

Table (7): Inter group Comparison # in DP/DT /mmHg/sec at end of reperfusion

Dependent variable	Group(I)	Group (J)	Mean difference (I - J)	*P* value
ΔP/Δ t	1b (control group)	2	63.0	0.005*
		3	-80.0	0.001*
		4	-99.0	0.000*
	2 (DPP-4 Inhibitor group	1	-63.0	0.005*
		3	-143.0	0.000*
		4	-162.0	0.000*
	3 (GLP-1 low dose group)	1	80.0	0.001*
		2	143.0	0.000*
		4	-19.0	0.378†
	4 (GLP-high dose	1	99.0	0.000*

	group)	2	162.0	0.000*
		3	19.0	0.378†

#Post-Hoc test was done * Significant difference (p<0.05) † Insignificant difference

Table (7): explains that there was significant difference between groups 1b&2 as regard DP/DT at end of reperfusion ***p* < 0.05**.

There was no significant difference between group 3&4 as regard DP/DT at end of reperfusion ***p* < 0.05**.

There was significant difference between group 1b& 3 as regard DP/DT at end of reperfusion ***p* < 0.05**.

There was no significant difference between group 1b&4 as regard DP/DT at end of reperfusion ***p* < 0.05**.

There was no significant difference between group 2&3 as regard DP/DT at end of reperfusion ***p* < 0.05**.

There was no significant difference between group 2&4 as regard DP/DT at end of reperfusion ***p* < 0.05**.

Table (8): Percentage decrease in mean values of cardiac parameters in all groups.

	Group 1b	Group 2	Group 3	Group4
B/PM	27.2%*	29.6%*	13.2%*	12.7%*
LVDP	44.0%*	51.0%*	31.5%*	33.5%*
RPP	59.6%*	65.3%*	46.6%*	42.0%*
ΔP/ΔT max	41.5%*	48.4%*	37.5%*	35.8%*

* Significant difference (p<0.05)

Table (8) explains that there was Significant differences between different groups in Percentage decrease of mean values of cardiac parameters which calculated by subtracting result 2 from result 1 and divided by result 1 multiplied by 100.

The highest decrease of cardiac parameters was shown in groups (1b & 2). The lowest decrease of cardiac parameters in groups 3 & 4 (GLP-1 groups).

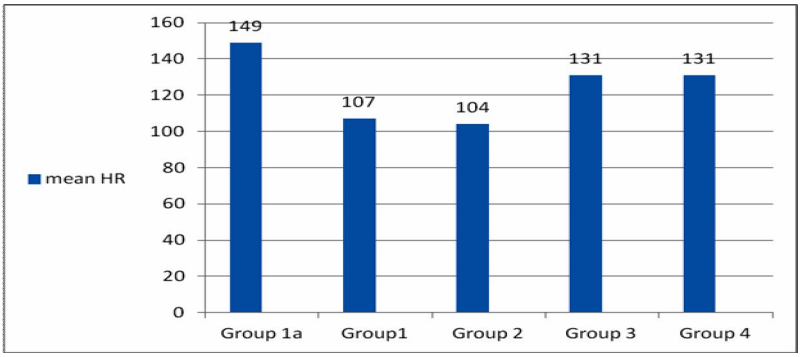


Figure (1): Mean heart rate (HR) in the five groups

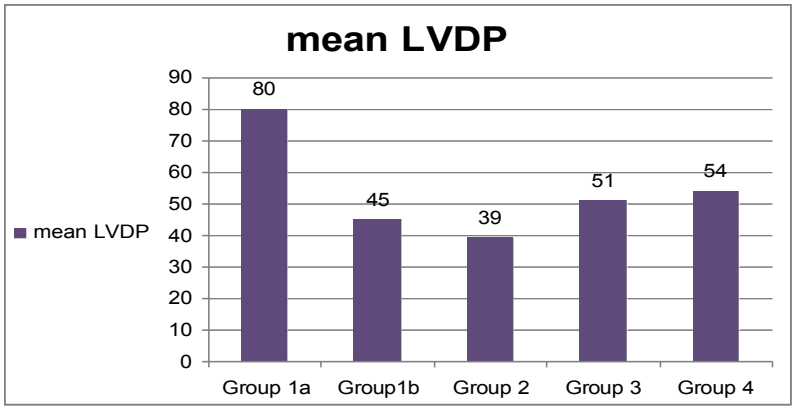


Figure (2): Mean left ventricular developed pressure (LVDP) in the five groups

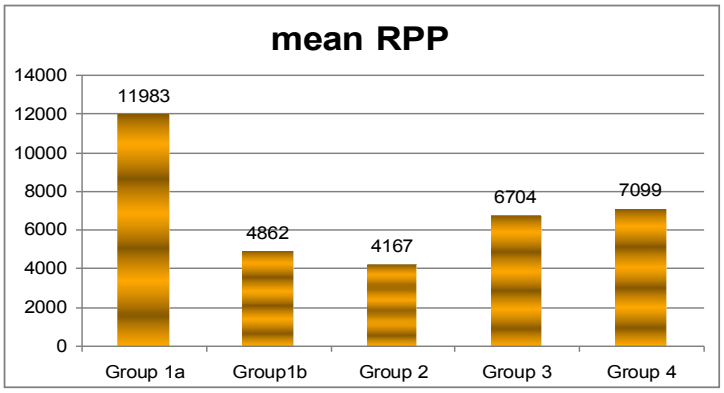


Figure (3): Mean rate pressure product (RPP) in the five groups.

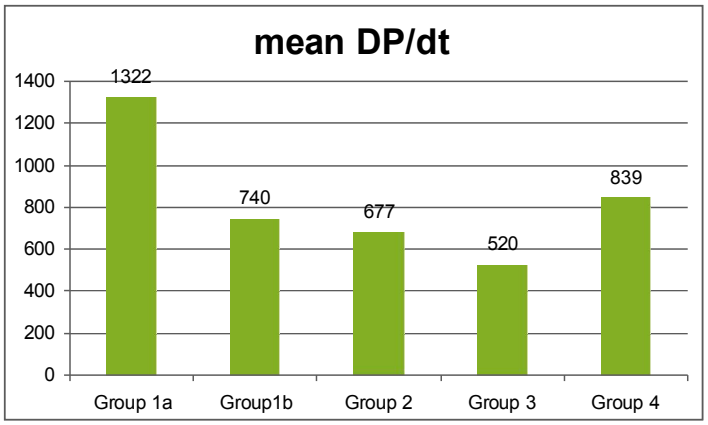


Figure (4): Mean value of (ΔP/ΔT) in the five groups.

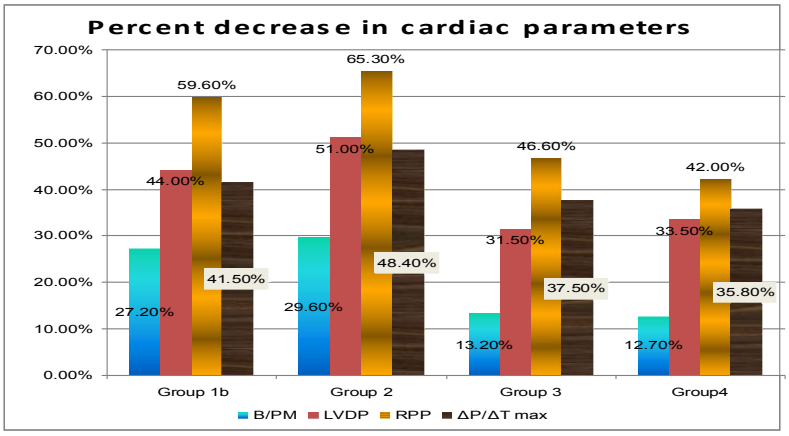


Figure (5): Percentage decrease in mean values of cardiac parameters in all groups.

Table (9): Comparison between groups# in infarction size.

Infarction size	Group 1b	Group 2	Group 3	Group 4
Median	55%	52.5%†	25%*	20%*
IQR	50 - 60	50 – 60	18.75-25	22 - 25

(Krustal wallis test was done) * Significant difference (*p*<0.05) † Insignificant difference

Table (9) explains that there are statistical difference between groups (1b&3) and between (1b&4) in infarction size and no statistical difference between groups (1b&2)

Histopathological results and determination of infarction size

The histopathological examination revealed the features of apoptosis in the form of juxtanuclear vacuolization of myocardial cells. The sarcoplasm exhibited granular degeneration with microfibrillar fragmentation (autolytic cell death) and lipofuscin deposits. Some myocardial cells showed increased cytoplasm acidophilia with nuclear pyknosis and polymorphonuclear neutrophiles infiltration. Edema was present in the interstitium, more prominent in the subendocardium.

In summery, changes occurred in infracted tissues are:

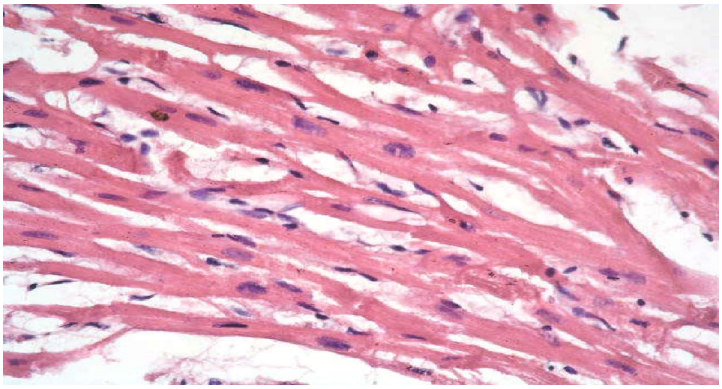
1-The myocytes show degeneration consisting of rarefaction of the cytoplasm with mild cytomegaly .

2- The nuclei show pyknosis, karyorrhexis and karyolysis.

3- The infracted area appears homogenous structure less and eosinophilic.

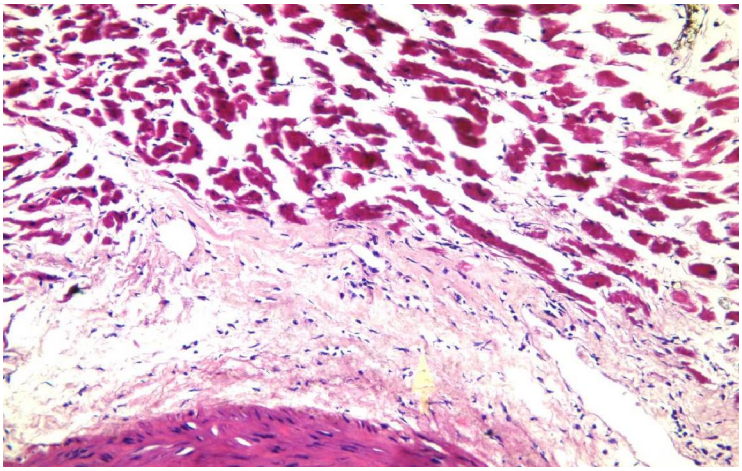
These changes more prominent in groups 1&2 and less in glp-1 groups (groups 3& 4) indicating cardio protective effect of glp-1.

Group 1 a

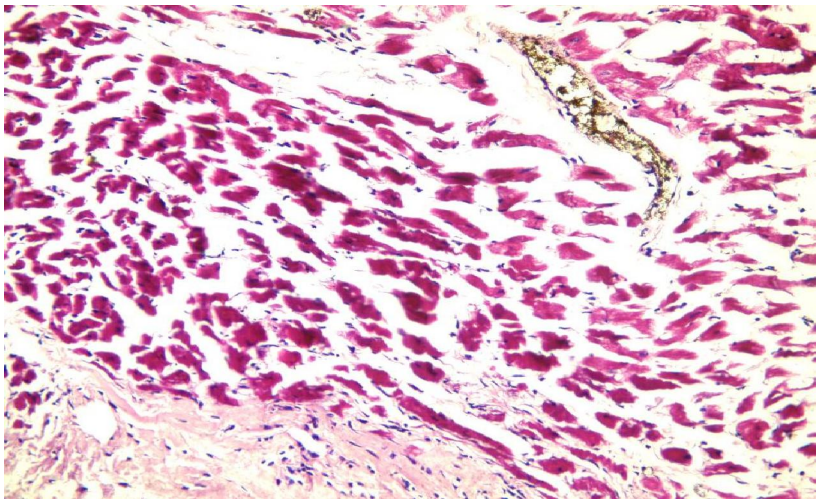


Picture(1): Section in the cardiac muscle of control group(non ischemic) showing normal cardiac muscle fibers which appears cylindrical branching with central ovale nuclei and acidophilic cytoplasm spreated by minimal amount of connective tissue.

Group 1b

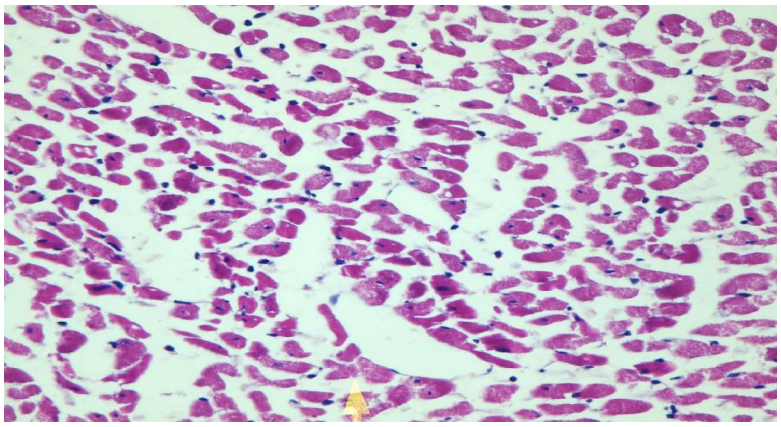


Picture (2): Section in the cardiac muscle of control group(ischemic) showing rarefaction of cytoplasm , nuclear pyknosis and healing by fibrous tissue

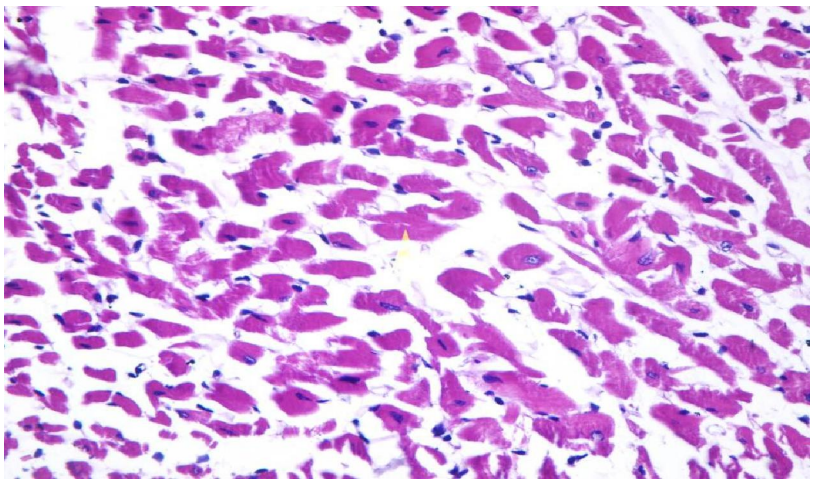


Picture (3): Section in the cardiac muscle of control group (ischemic) showing rarefaction of cytoplasm , nuclear pyknosis and healing by fibrous tissue

Group 2

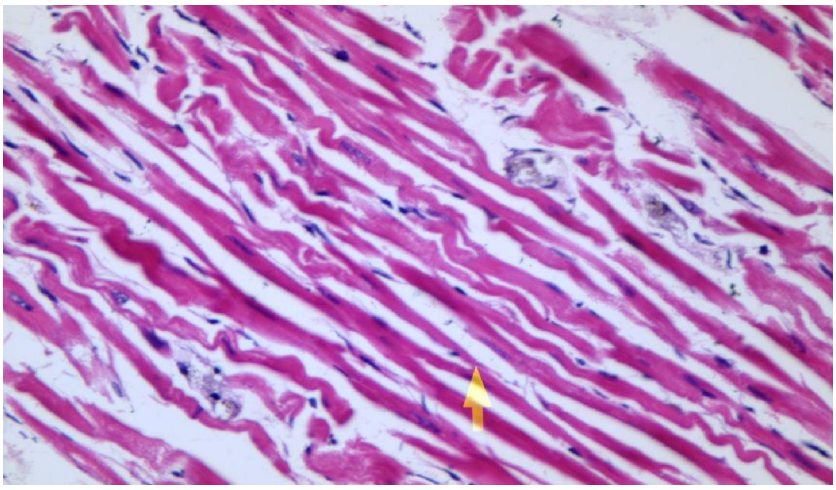


Picture (4): Section in the cardiac muscle of sitagliptin group (ischemic) showing rarefaction of cytoplasm , nuclear pyknosis.



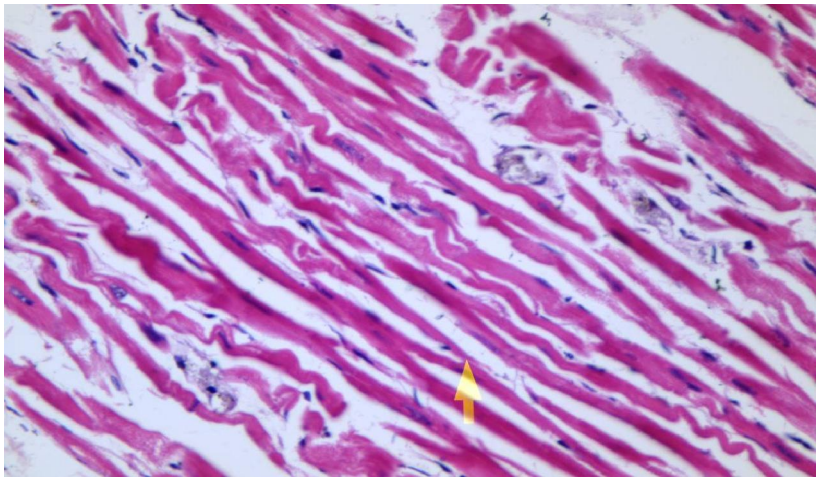
Picture (5): Section in the cardiac muscle of sitagliptin group (ischemic) showing rarefaction of cytoplasm , nuclear pyknosis .

Group 3



Picture (6): Section in the cardiac muscle of GLP-1 small dose group showing normal myocytes with normal nuclei, minimal fibrous tissue.

Group 4



Picture (7): Section in the cardiac muscle of GLP-1 high dose group showing normal myocytes with normal nuclei, minimal fibrous tissue , no rarefaction of cytoplasm ,no nuclear pyknosis& no healing by fibrosis

4. Discussion

In the present study sitagliptin alone (group2) has no effect in cardiac performance as shown in tables (4,5,6,7) i.e. significant reduction in heart rate, ventricular developed pressure and contractility due to loss of cardiomyocytes with no improvement in functional recovery as shown in table(13). This result was agreed with green *et al*[10] . The above result not agreed With **Ye *et al.*** [11] (11) who found that sitagliptin seemed to improve functional recovery from ischaemia/reperfusion in mice and presented similar cardioprotection with genetic deletion of DPP-4 . Sitagliptin has also been associated with a reduction in infarct size in these experimental models(11).

It is clear from table (3) that functional recovery of cardiac parameters was higher in GLP-1 treated groups (3&4) this result was agreed by(12). Recovery of left ventricular developed pressure (LVDP) and contractility (ΔP/ΔT max) is a good indication of inotropic effect of GLP-1. An agent's total effect on myocardial performance in a postconditioning paradigm is a sum of its myocyte - preserving (cardioprotective) and contractility-affecting (negative or positive inotropic) action components.

HR is significantly higher in glp-1treated groups in the present study i.e. GLP-1 has inotropic & chronotropic effect which agreed by **Yamamoto *et al.*, & Anagnostis *et al.*** [13,14]

However, other investigators failed to confirm such haemodynamic effects in pigs(15), while others reported negative inotropic effects of GLP-1 on rat cardiomyocytes *in vitro*(16) .

In contrary **Thrainsdottir *et al*.**[17] found that both short- and long-term administration of GLP-1 in humans have been found to have no detectable chronotropic or pressor effects .These findings may suggest a species-specific effect of GLP-1, although it should be noted that the bigger animal and human studies generally employed lower concentrations of GLP-1, within the low picomolar range, compared to the rodent studies which mostly administered large supra-physiological doses given as bolus injections.

As a result of increased HR& LVDP there was significant increase RPP in GLP-1 treated groups compared to control& sitagliptin groups as shown in table (6)

Table (8) shows that there was marked decrease in cardiac parameters occurred in groups (1b & 2) with less decrease in GLP-1 groups (3 & 4). This confirm the role of GLP-1 in cardioprotection in low and high doses.

Histopathological results showing significant reduction in infarction size by 50% in GLP-1 treated groups compared to control& sitagliptin groups as shown in table (9). This was proved by the majority of studies on the potential beneficial role of GLP-1 in CVD that focused on its actions in the ischaemic heart and its apparent ability to protect cardiac myocytes from ischaemic damage. Several different groups using various experimental models have reported that acute GLP-1 treatment exerts beneficial effects after ischaemia and successful reperfusion. Most of the studies to date have employed models of ex vivo isolated rodent Langendorff heart perfusion with short periods of ischaemia (30–45 min) and reperfusion (30–120 min), and have universally demonstrated that both GLP-1 and exendin-4 significantly reduce infarct size and enhance the recovery of contractile function after transient coronary artery occlusion[7,14, 19,19 & 20].

Although evidence from multiple studies suggests that GLP-1 has important cardiovascular actions, the mechanisms underlying these diverse effects had not been fully elucidated. These results propose a novel two-pathway schema for cardiovascular actions of GLP-1, one (A) which depends on the GLP-1R for (i) inotropic action, (ii) glucose uptake, (iii) ischemic pre-conditioning and (iii) mild vasodilatory actions, and the second (B) which depends on rapid metabolism of GLP-1 to GLP-1(9-36), the latter having GLP-1R-independent effects on (i) post-ischemic recovery of cardiac function, and (ii) vasodilation. other results also suggest that GLP-1(9-36) is (a) not an inotrope, (b) has at best modest effects on myocardial glucose uptake in vitro, and (c) causes vasodilatation through a NO/cGMP-dependent mechanism, which also participates in cardioprotective effects in the setting of I/R injury[21].

Acute treatment with GLP-1 (in the presence of the DPP-4 inhibitor, valine pyrrolidide) after a short period of ischaemia (30 min) in the rat was found to significantly protect against infarct development after a 2 h reperfusion[18].

GLP-1 also attenuates myocardial stunning and reduces infarct size after ischemia-reperfusion in conscious dogs and anesthetized rats, respectively. Moreover, studies using isolated heart preparations have shown that GLP-1 has direct protective effects on the heart. GLP-1 reduces infarct size and increases left ventricular function and myocardial glucose uptake after ischemia-reperfusion injury in isolated rat hearts[8].

The protective effects of GLP-1 in these studies are mediated by cAMP and the pro survival kinases PI-3K/Akt and p44/42 MAPK.GLP-1R signaling is essential for normal cardiac structure and function as GLP-1R-/- mice exhibit increased septal and postero lateral myocardial wall thickness and abnormal cardiac contractile responses to external stresses[22] .

GLP-1 induces an increased level of cAMP in cardiomyocytes[16], which, in turn, activates protein kinase A. GLP-1 has an antiapoptotic action on insulin-secreting cells mediated by cAMP and PI3K[5]. Activation of PI3K leads to the phosphorylation and inactivation of the proapoptotic peptide BAD by causing it to bind to 14-3-3 proteins[23]. BAD is a proapoptotic member of the Bcl-2 family that can displace Bax from binding to Bcl-2 and Bcl-xl, resulting in cell death.

The exact mechanisms underlying cardioprotective effect of GLP-1 have not been fully elucidated. First of all, GLP-1 increases myocardial insulin sensitivity[24], as well as myocardial glucose uptake independently of plasma insulin levels[25].

Moreover, the survival of cardiac myocytes is mediated by inhibition of apoptosis via cAMP and PI3-K pathways, after binding with GLP-1Rs[18]. Furthermore, the activation of the antioxidant gene, hemeoxygenase-1 (HO-1), through GLP-1 reduces fibrosis and LV remodelling and restores LV function after MI[26].

HO-1 acts via induction of nuclear factor-E2-related factor (Nrf)2 gene expression and nuclear translocation and subsequent stimulation of Akt[27]. Other cardioprotective mediators are glycogen synthase kinase (GSK)-3β, Bcl-2 family proteins[28] and PPARs-β and -δ[29]

Nevertheless, GLP-1 action is also mediated throughGLP-1R-independent pathways. In particular, under the influence of DPP-4, GLP-1(7-36) amide is degraded to the inactive N-terminally truncated metabolite GLP-1(9-36) amide, which does not interact with the known GLP-1R[30]. Data from isolated mouse heart models show that GLP-1(9-36)

exerts a vasodilatory effect through a GLP-1R-independent mechanism via the formation of cyclic guanosine monophosphate (c GMP) by nitric oxide (NO) which, in turn, is produced under the action of nitric oxide synthase (NOS).Native GLP-1 as well as the synthetic analogue exendin-4 which is DPP-4 resistant and therefore cannot be metabolized to GLP-1(9-36), improve LV functional recovery after ischaemia–reperfusion injury. However, for animals lacking GLP-1Rs, this action was evident only for GLP-1 and not for exendin-4[19]

Moreover, GLP-1 and not GLP-1(9-36) displayed a direct inotropic action via GLP-1R in the mouse heart and vasculature. The GLP-1R-independent role of GLP-1(9-36) for the cardiovascular system was further indicated from a study of conscious dogs with dilated cardiomyopathy, in which infusions of GLP-1(9-36) improved LV function and increased myocardial glucose uptake[31]

Noticeably, another experimental rat model evaluating the effects ofGLP-1(7-36) on the cardiovascular system and elucidating the role of GLP-1(9-36) showed that GLP-1(7-36) infusion was characterized by regional haemodynamic effects including tachycardia, hypertension, renal and mesenteric vasoconstriction, whereas GLP-1(9-36) did not display any cardiovascular actions.

It can be concluded from the present study that GLP-1 has acardioprotective role even during myocardial ischemia thus GLP-1 represent a logical and effective treatment for diabetes to prevent diabetic cardiomyopathy.

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References:

1- Drucker DJ (2006). The biology of incretin hormones. Cell Metab 3: 153–165.
2- Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R (1992). Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22: 283–291.
3- Baggio LL, Drucker DJ (2007). Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131–57.
4- Deacon CF (2004).Circulation and degradation of GIP and GLP-1. Horm Metab Res 36: 761–765.
5- Hupe-Sodmann K, Goke R, Goke B, Thole HH, Zimmermann B, Voigt K (1997). Endoproteolysis of glucagon-like peptide (GLP)-1(7– 36) amide by ectopeptidases in RINm5F cells. Peptides 18: 625–632.
6- Yellon DM, Dana A (2000). The preconditioning phenomenon: a tool for the scientist or a clinical reality? Circ Res.87: 543–550.
7- Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima IG, Shen YT (2006).The direct effects of glucagon-like peptide-1 (GLP-1) on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. J Pharmacol Exp Ther 317: 1106–1113.
8- Bose A, Mocanu M, Carr R, Yellon D (2007). Myocardial ischaemia– reperfusion injury is attenuated by intact glucagon like peptide-1 (GLP-1) in the in vitro rat heart and may involve the p70s6K pathway. Cardiovasc Drugs Ther 21: 253–256.
9- Alvilde Ossuma, Ulla van Deursa, Thomas Engstr̈mb, Jan Skov Jensenc, Marek Treimana (2009). The cardioprotective and inotropic components of the postconditioning effects of GLP-1 and GLP-1(9–36)a in an isolated rat heart. Pharmacological Research 60 411–417.
10- Green BD, Flatt PR, Bailey CJ (2007). Dipeptidyl peptidase IV (DPP IV) inhibitors: a newly emerging drug class for the treatment of type 2 diabetes. Diab Vasc Dis Res 3: 159–165.
11- Ye Y, Keyes KT, Zhang C (2010). The myocardial infarct size-limiting effect of sitagliptin is PKA-dependent, whereas the protective effect of pioglitazone is partially dependent on PKA. Am J Physiol Heart Circ Physiol 298: H1454–H1465.
12- Okerson T, Yan P, Stonehouse A, Brodows R (2010). Effects of exenatide on systolic blood pressure in subjects with type 2 diabetes. Am J Hypertens 23: 334–339.
13- Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME (2002). Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J Clin Invest 110: 43–52.
14- Anagnostis P, Athyros V. G. , Adamidou F &Mikhailidis D. P. (2011). Glucagon-like peptide-1-based therapies and cardiovascular disease: looking beyond glycaemic control. Diabetes, Obesity and Metabolism 13: 302–312.
15- Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ (1996). Glucagonlike peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. Am J Physiol Endocrinol Metab 271: E458–E464.
16- Vila Petroff MG, Egan JM, Wang X, Sollott SJ (2001). Glucagon-like peptide-1 increases

cAMP but fails to augment contraction in adult rat cardiac myocytes. Circ Res 89: 445–452.
17- Thrainsdottir I, Malmberg K, Olsson A, Gutniak M, Rydén L (2004). Initial experience with GLP-1 treatment on metabolic control and myocardial function in patients with type 2 diabetes mellitus and heart failure. Diab Vasc Dis Res 1: 40–43.
18- Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM(2005a). Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. Diabetes 54: 146–151.
19- Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M (2008). Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 117: 2340–2350.
20- Sonne DP, Engstrom T, Treiman M (2008). Protective effects of GLP-1 analogues exendin-4 and GLP-1(9–36) amide against ischemia–reperfusion injury in rat heart. Regul Pept 146: 243–249.
21- Kloner RA, JenTetsuo M., Masafumi K., nings RB (2001). Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 2. Circulation. 18;104(25):3158-3162
22- Gros R, You X, Baggio LL, Kabir MG, Sadi AM, Mungrue IN (2003). Cardiac function in mice lacking the glucagon-like peptide-1 receptor. Endocrinology 144: 2242–2252.
23- Best JH, Hoogwerf BJ, Herman WH (2011). Risk of cardiovascular disease events in patients with type 2 diabetes prescribed the GLP-1 receptor agonist exenatide twice daily or other glucose-lowering therapies: a retrospective analysis of the LifeLinkTM Database. Diabetes Care 34: 90–95.
24- Nikolaidis LA, Mankad S, Sokos GG, Miske G, Shah A, Elahi D (2004b). Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. Circulation 109: 962–965.
25- Bhashyam S, Fields AV, Patterson B (2010). Glucagon-like peptide-1 increases myocardial glucose uptake via p38 alpha MAP kinase-mediated, nitric oxide-dependent mechanisms in conscious dogs with dilated cardiomyopathy. Circ Heart Fail 3: 512–521.
26- Yin F, Liu JH, Zheng XX, Guo LX (2010). GLP-1 receptor plays a critical role in geniposide-induced expression of heme oxygenase-1 in PC12 cells. Acta Pharmacol Sin 31: 540–545.
27- Piantadosi CA, Carraway MS, Babiker A, Suliman HB (2008). Heme oxygenase- 1 regulates cardiac mitochondrial biogenesis via Nrf2-mediated transcriptional control of nuclear respiratory factor-1. Circ Res 103: 1232–1240.
28- Juhaszova M, Zorov DB, Yaniv Y, Nuss HB, Wang S, Sollott SJ (2009). Role of glycogen synthase kinase-3beta in cardioprotection. Circ Res104: 1240–1252.
29- Burkart EM, Sambandam N, Han X. (2007). Nuclear receptors PPARbeta/ delta and PPARalpha direct distinct metabolic regulatory programs in the mouse heart. J Clin Invest 117: 3930–3939.
30- Ban K, Kim KH, Cho CK (2010). Glucagon-like peptide (GLP)-1(9-36) amide mediated cytoprotection is blocked by exendin (9-39) yet does not require the known GLP-1 receptor. Endocrinology 151: 1520–1531.
31- Nian M, Drucker DJ, Irwin D (1999). Divergent regulation of human and rat proglucagon gene promoters in vivo. Am J Physiol 277: G829–G837.

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