Effect of Acute Apelin Injection on Cardiac Muscle Performance in both Normal and Diabetic Rats

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Abstract: Background: Apelin is an adipokine originally identified as the endogenous ligand of the G protein coupled receptor APJ. Several studies have demonstrated that apelin and its receptor are involved in the regulation of cardiovascular function. Apelin was also found to have a positive inotropic effect in both rat and human hearts. However, this effect in case of cardiovascular diseases is controversial. Diabetes mellitus is one of the major risk factors for cardiovascular disease which is the leading cause of death in those patients**. Aim**: This study was designed to detect possible acute effects of *in vivo* apelin-13 injection on cardiac performance in both normal and diabetic state, with a trial to clarify possible involved mechanisms .**Material & methods**: This study was conducted on 72 healthy, adult, male albino rats. The animals were divided equally into three main groups: **Group I:** Control group. **Group II:** Streptozotocin -induced diabetic non treated rats. **Group III**: Insulin treated diabetic rats. **Experimental design:** In the three groups we examined the effect of acute injection of apelin-13 (10 nmol /kg b.wt) alone or in the presence of propranolol (0.2mg/kg b.wt), verapamil (4.8mg/kg), benzamil HCL (Na/ Ca^{+2} exchange (NCX) blocker) (10 nmol/kg), on cardiac muscle performance. **Results**: The present results demonstrated that apelin-13 administration significantly increased cardiac muscle performance ($p<0.001$) without any significant changes in heart rate, in all groups, as evidenced by the significant increase in ($+dT_{max}/t_{max}$) and ($-dT_{max}/t_r$). In addition, this increase was more significant in diabetic rats in comparison with that of both control and diabetic treated rats. Moreover, the observed effects are independent of the voltage- gated calcium channels or B- adrenergic receptors but appear to involve activation of the sacrolemmal Na^{++} /Ca^{++} exchanger (NCX). **Conclusion:** apelin-13 exerted both positive inotropic and lusiotropic effects without affection of the heart rate in vivo, which was more significant in diabetic rats in comparison with that of both normal and insulin treated rats. Our results also suggested that this response to apelin involved activation of Na^{+}- Ca^{+2} exchange channels (NCX). Therefore, the use of apelin may be investigated as a potential therapeutic target for diabetic cardiomyopathy. However, the impact of chronic administration requires further attention.
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1. Introduction

Apelin is a bioactive peptide originally identified from bovine stomach extracts as the endogenous ligand of the G protein coupled receptor

APJ **(Boucher *et al.*, 2005)**. Apelin is considered as an adipokine, essentially as the result of its increased expression during adipocyte differentiation and its release by differentiated adipose cells into the medium culture (**Wei *et al.*, 2005**).

The next studies have demonstrated that apelin and its receptor are widely expressed in the central nervous system (CNS) and peripheral tissues, and involved in the regulation of cardiovascular function **(Hosoya *et al.*, 2000, Lee *et al.*, 2000, Kawamata *et al.*, 2001 , Macaluso *et al.*, 2011).**

Most importantly, apelin has been shown to act as an endogenous inotrope regulating cardiac contractility (**Ashley *et al.*, 2005, Jia *et al.*, 2006, Zeng *et al.*, 2007)** and playing an important role in paracrine signaling in the heart (**Chen *et al.*, 2003 and Földes *et al.*, 2003**).

Diabetes mellitus is one of the major risk factors for cardiovascular disease which is the leading cause of death in those patients**.** Aside from large vessel disease and accelerated atherosclerosis, which is very common in diabetes, diabetic cardiomyopathy is a clinical condition diagnosed when ventricular dysfunction develops in patients with diabetes in the absence of coronary atherosclerosis and hypertension **(Avogaro *et al.*, 2004).**

Apelin expression in adipose tissue is regulated by nutritional status, such as fasting and refeeding **(Boucher *et al.*, 2005)**, insulin **(Wei *et al.*, 2005)** and tumor necrosis factor- alpha **(Daviaud *et al.*, 2006)**. Mice with streptozotocin-induced diabetes mellitus had decreased apelin expression **(Boucher *et al.*, 2005)**, whereas apelin levels were increased in obese, hyperinsulinemic humans compared to normal weight subjects **(Heinonen *et al.*, 2005 and Boucher *et al.*, 2005)**.

In addition, accumulating evidence supports apelin involvement in cardiovascular function, but its

causative relationship with ischemic heart disease is controversial **(Ronkainen *et al.,* 2007, Chandrasekaran *et al.,* 2008 and Rastaldo *et al.,* 2011).** Limited evidence has emerged, indicating the association of reduced apelin with coronary atherosclerosis **(Weir *et al.,* 2009).** Consistent with previous studies of **Li *et al.* (2008), Kadoglou *et al.* (2010)** found lower apelin levels in patients with coronary artery diseases (CAD) than in the healthy controls. Besides this finding, they confirmed the correlation of low apelin concentrations with a CAD presence and severity. Moreover, lower plasma apelin was associated with left ventricular systolic and diastolic function impairment **(Przewlocka-Kosmala *et al.,* 2011)**

Importantly, the latter relationship was independent of other traditional cardiovascular risk factors. Taken together, apelin emerged as a novel biomarker of coronary atherosclerosis development and severity, but this result remains to be proved prospectively **(Kadoglou *et al.,* 2010)**.

Up to our knowledge, there is no information on the functional in vivo effects of apelin in case of diabetic cardiomyopathy. Moreover, the possible mechanisms of action of apelin on cardiac performance have not yet been sufficiently cleared.

This study was designed to detect possible acute in vivo effects of apelin on cardiac performance in both normal and diabetic state, with a trial to clarify possible involved mechanisms.

2. Animals and methods

Animals:

This study was conducted on 72 healthy, adult, male albino rats weighing 180- 200 gm. The animals had free access to water and chow and were kept at room temperature.

Ethical committee approval for the study was obtained from Zagazig University

The animals were divided equally into 3main groups:

Group I: To study the acute effect of apelin-13 injection (10 nmol /kg) **(Cheng et al., 2003)** on cardiac muscle performance of normal rats.

Group II: To study the acute effect of apelin-13 injection (10 nmol /kg) **(Cheng et al., 2003)** on cardiac muscle performance of strptozotocin -induced type 1 diabetic non treated rats.

Diabetes was induced by a single intra-peritoneal injection of freshly prepared solution of streptozotocin 65 mg/kg of body weight dissolved in 0.2 mmol/L sodium citrate, at pH 4.5 **(Lutz and Pardridge, 1993)** and the rats maintained for 6 weeks **(Srinivasan et al., 1997, Shenoy and Goyal 2002)**.

Three days later, diabetes induction was confirmed through measurement of blood glucose level in each animal (blood was sampled from the tail vein) with the One Touch Ultra Glucometer **(Yves and Theo, 2007)** and rats with blood glucose levels more than 250 mg/dl were selected for experiments **(Coskun et al., 2004)**. The rats were provided with oral 10% glucose solution after 6 hours of streptozotocin administration for the next 48 hours.

Group III: To study the acute effect of apelin-13 injection (10 nmol /kg) on cardiac muscle performance of strptozotocin -induced type 1 diabetic insulin treated rats. These animals were treated with regular (R) and NPH (N) insulin (2UR at diagnosis of diabetes and then 1R/3N at 6 P.M and 1R/1N at 9 A.M daily subcutaneously for 6 weeks after induction of diabetes **(Sivitz et al., 1998).**

Methods:

Recording of cardiac muscle performance parameters via D1 isometric transducer (Bioscience, London) attached to **a** 4-channel oscillograph "MD4" (Bioscience, London)

The rats were anaesthetized by intraperitoneal injection of ethyl carbamate (urethane) in a dose 1.75-2 gm /kg body weight injected intraperitonealy as 25 % freshly prepared aqueous solution **(Gosh, 1971).** Tracheotomy was performed on the neck to open a direct airway through an incision in the trachea and connected to the artificial ventilator. The rats were ventilated with room air at 60-70 breaths/ min. The right jugular vein was cannulated to infuse saline or drugs throughout the experiment. Upon completion of the surgical procedures, the animals were allowed to stabilize, generally for 30 min.

A 6-0 prolene suture was fixed to the ventricle and passed via thoracotomy to be attached to the hock of D1 isometric transducer (the baseline tension of the rat heart is adjusted at 2.00 grams). The FC 117 direct input coupler is fixed to one channel of the oscillograph and connected to D1 isometric transducer. Calibration of the isometric transducer using increasing weights, and recording the corresponding pen deflection was done before starting anesthesia.

Experimental design

Experiment I: to study the acute effect of apelin-13 injection (10 nmol/kg) **(Cheng et al., 2003)** on cardiac muscle performance in the three main groups (n=18)

Experiment II: to study the acute effect of apelin-13 injection (10nmol/kg) on cardiac muscle performance 10 minutes after the propranolol injection (0.2mg/kg) **(Vongpatanasin et al., 1999)** in the three main groups (n=18)

Experiment III: to study the acute effect of apelin-13 (10 nmol/kg) on cardiac muscle performance

10minutes after the verapamil (ca $^{+2}$ channel blocker) injection (4.8mg/kg) **(Persson et al., 2007)** in the three main groups (n=18).

Experiment IV: to study the acute effect of apelin-13 injection (10nmol/kg) **(Cheng et al., 2003)** on cardiac muscle performance 10 minutes after benzamil HCL (Na/ Ca^{+2} exchange (NCX) blocker) injection (10 nmol/kg) **(Nishimura et al., 1998)** in the three main groups (n=18).

NB: The maximal effect of apelin injection on cardiac muscle performance was calculated and statstatically investigated in all experiments (this effect was about 5-10 minutes after its injection).

Calculation of the studied parameters

1-Maximum tension developed ($+dT_{max}$): It was obtained from the calibration of tension on the graph in grams.

2-Time to reach maximum tension (t_{max}): From the point of maximum tension a vertical was drawn to meet the baseline of the recorded tension on the graph. The distance on the baseline from the onset of tension rise till vertical line was measured. As the speed of the oscillograph equal to 50 mm/ sec, so every 1mm measured on the baseline equal to 0.02sec. According to the latter equation the time to reach maximum tension **(t_{max})** was calculated in seconds.

3- Rate of developing tension ($+dT_{max}/ t_{max}$): By dividing Maximum tension developed **($+dT_{max}$)** by time to reach maximum tension **(t_{max})** was calculated as gm/ sec.

4-Time of cardiac relaxation (t_r): It was calculated by measuring the distance on the baseline from the point of maximum tension till return to basal tension. The time of cardiac relaxation was assessed as every 1mm equals 0.02 sec.

5-Rate of cardiac relaxation ($-dT_{max}/t_r$): By dividing the maximum tension developed by the time of cardiac relaxation **(t_r)**; the rate of cardiac relaxation **($-dT_{max}/t_r$)** was calculated and expressed as gm/sec.

6- Calculation of the heart rate/ minute was carried out by counting the number of the heart cycles (n) per fixed distance of chart paper **(Gay, 1965)**.

Statistical analysis:

Data were presented as mean ± SD. Statistical significance was determined by one way analysis of variance (ANOVA) between the three main groups, and student's t test (paired and unpaired) in the same group. P values less than 0.05 were considered to be significant. In statistical analysis, SPSS program version 10.0 for Windows (SPSS Inc. Chicago, IL, USA) was used**.**

3. Results

Table 1: Shows blood glucose levels (mg/dl) at the end of the study period in all groups. Serum glucose levels in group II (mean ± SD) (413.5 ± 85.49mg/dl) was significantly increased ($P< 0.001$) when compared with that of group I (78.1 ± **6**.32mg/dl). Moreover, in group III serum glucose levels were significantly decreased and return to the normal levels when compared with that of group II (81.77 ± 5.88mg/dl& $P < 0.001$).

Table 2 and record 1: Show cardiac contractility parameter; the rate of development of tension (+dTmax/tmax) [gram/second] and cardiac relaxation parameter; rate of relaxation (-dTmax/ tr) [gram/second] and heart rate in the three main groups: There was a significant decrease in (+dTmax/tmax)[gram/second] (mean± SD) (91.7± 5.2 gram/second) in diabetic group in comparison with that of both Control (110.3± 12.8 gram/second, $P<0.01$) and insulin treated (104.8± 10.7 gram/second, $P<0.05$) groups.

In addition, there was a significant decrease in (-dTmax/ tr) [gram/second]: (mean± SD) (31.2± 3.6 gram/second) in diabetic group in comparison with that of both control (36.1± 2.3 gram/second, $P<0.01$) and insulin treated groups (36.8± 0.8 gram/second, $P<0.01$).

In addition, there was a significant decrease in heart rate (mean± SD) (320± 15.5 beat\ min) in diabetic group in comparison with that of both control (355± 22.6 beat\ min, $P<0.05$) and insulin treated (350± 31 beat\ min, $P<0.05$) groups.

Table 3 and record 2: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac contractility parameter; the rate of development of tension (+dTmax/tmax) [gram/second] in the three main groups.

In group I: there was a significant ($P< 0.001$) increase in (+dTmax/tmax) from (mean± SD) (110.3± 12.8 gram/second) to (133.3± 12.9 gram/second) after apelin injection.

In group II: there was a significant ($P< 0.001$) increase in (+dTmax/tmax) from (mean± SD) (91.7± 5.2 gram/second) to (120.5± 5.8 gram/second) after apelin injection.

In group III: there was a significant ($P< 0.001$) increase in (+dTmax/tmax) from (mean± SD) (104.7± 10.7 gram/second) to (127,5± 10 gram/second) after apelin injection.

Moreover, the percentage of increase was more significant in diabetic group (group II), (mean± SD) was (31.5±2.6) compared to that of both group I (21.2±3.1, $P < 0.001$) and group III (20.2±3, $P < 0.001$).

Table 4 and record 2: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac

relaxation parameter; rate of relaxation (-dTmax/tr) [gram/second] in the three main groups.

In group I: there was a significant (P< 0.001) increase in (-dTmax/tr) from (mean± SD) (36.1± 2.3 gram/second) to (44.8± 3.1 gram/second) after apelin injection.

In group II: there was a significant (*P*< 0.001) increase in (-dTmax/tr) from (mean± SD) (30.8±2.8 gram/second) to (40.5± 2.9 gram/second) after apelin injection.

In group III: there was a significant (*P*<0.001) increase in (-dTmax/tr) from (mean±SD) (37±1 gram/second) to (46.1±1.2 gram/second) after apelin injection.

Moreover, the percentage of increase was more significant in diabetic group (group II), (mean±SD) was (29.8±3.2) compared to that of both group I (23.1±4.1, P<0.01) and group III (25.5±3, P<0.05).

Table 3 and record 3: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac contractility parameter; the rate of development of tension (+dTmax/tmax) [gram/second] in the presence of verapamil (4.8 mg/kg) in the three main groups.

In group I: there was a significant (*P*<0.001) increase in the rate of development of tension (+dTmax/tmax) after apelin-13 injection in the presence of verapamil, (mean± SD) was (94.3± 9.3 gram/second) compared to (79± 7.7 gram/second) before apelin-13 injection.

In group II: there was a significant (P<0.001) increase in the rate of development of tension (+dTmax/tmax) after apelin-13 injection in the presence of verapamil, (mean± SD) was (83.8± 13 gram/second) compared to (64.2± 9.3 gram/second) before its injection.

In group III: there was a significant (P<0.01) increase in the rate of development of tension (+dTmax/tmax) after apelin-13 injection in the presence of verapamil, (mean± SD) was (85.3± 9.6 gram/second) compared to (72.6± 7.1 gram/second) before its injection.

Furthermore, no significant difference was detected in the percentage of increase in (+dTmax/tmax) in the presence of verapamil in comparison to that produced by apelin alone in all groups.

Table 4 and record 3: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac relaxation parameter; rate of relaxation ($-dT_{max}/t_r$) [gram/second] in the presence of verapamil injection (4.8mg/kg) in the three main groups.

In group I: there was a significant (P<0.001) increase in the rate of relaxation ($-dT_{max}/t_r$) after apelin-13 injection in the presence of verapamil, (mean± SD) was (37.2± 2.5 gram/second) compared to (29.8± 1.4 gram/second) before apelin-13 injection.

In group II: there was a significant (P<0.001) increase in the rate of relaxation ($-dT_{max}/t_r$) after apelin-13 injection in the presence of verapamil, (mean± SD) was (35.5± 2.9 gram/second) compared to (27.4± 2.2 gram/second) before its injection.

In group III: there was a significant (P<0.001) increase in the rate of relaxation ($-dT_{max}/t_r$) after apelin-13 injection in the presence of verapamil, (mean± SD) was (38± 2.5 gram/second) compared to (30.7± 1.9 gram/second) before its injection.

Furthermore, no significant difference was detected in the percentage of increase in ($-dT_{max}/t_r$) in the presence of verapamil in comparison to that produced by apelin alone in all groups**.**

Table 3 and record 4: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac contractility parameter; the rate of development of tension ($+dT_{max}/t_{max}$) [gram/second] in the presence of propranolol injection (0.2mg/kg) in the three main groups.

In group I: there was a significant (P<0.001) increase in the rate of development of tension ($+dT_{max}/t_{max}$) after apelin-13 injection in the presence of propranolol, (mean± SD) was (91.5± 6.5 gram/second) compared to (76.3± 6.5 gram/second) before apelin-13 injection.

In group II: there was a significant (P<0.001) increase in the rate of development of tension ($+dT_{max}/t_{max}$) after apelin-13 injection in the presence of propranolol, (mean± SD) was (66.6± 4.5 gram/second) compared to (52± 3.7 gram/second) before its injection.

In group III: there was a significant (P<0.01) increase in the rate of development of tension ($+dT_{max}/t_{max}$) after apelin-13 injection in the presence of propranolol, (mean± SD) was (87.7± 7.2 gram/second) compared to (72.3± 6.4 gram/second) before its injection.

Furthermore, no significant difference was detected in the percentage of increase in ($+dT_{max}/t_{max}$) in the presence of propranolol in comparison to that produced by apelin alone in all groups.

Table 4 and record 4: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac relaxation parameter; rate of relaxation ($-dT_{max}/t_r$) [gram/second] in the presence of propranolol injection (0.2mg/kg) in the three main groups.

In group I: there was a significant (P<0.001) increase in the rate of relaxation ($-dT_{max}/t_r$) after apelin-13 injection in the presence of propranolol, (mean± SD) was (38.5± 2.4 gram/second) compared to (31.7± 1.8 gram/second) before apelin-13 injection.

In group II: there was a significant (P<0.001) increase in the rate of relaxation ($-dT_{max}/t_r$) after apelin-13 injection injection in the presence of propranolol, mean± SD) was (36.7± 1.3 gram/second)

compared to (28.2± 0.7 gram/second) before its injection.

In group III: there was a significant (P<0.001) increase in the rate of relaxation ($-dT_{max}/t_r$) after apelin-13 injection in the presence of propranolol, (mean± SD) was (37.8± 2.2 gram/second) compared to (30.2± 1.9 gram/second) before its injection.

Furthermore, no significant difference was detected in the percentage of increase in ($-dT_{max}/t_r$) in the presence of propranolol in comparison to that produced by apelin alone in all groups.

Table 3 and record 5: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac contractility parameter; the rate of development of tension ($+dT_{max}/t_{max}$) [gram/second]in the presence of benzamil hydrochloride injection (10 nmol/kg) in the three main groups.

In group I: there was a significant (P<0.01) increase in the rate of development of tension ($+dT_{max}/t_{max}$) after apelin-13 injection in the presence of benzamil hydrochloride, (mean± SD) was (120.3± 13.1 gram/second) compared to (111.8± 12.8 gram/second) before apelin-13 injection.

In group II: there was a significance (P<0.001) increase the rate of development of tension ($+dT_{max}/t_{max}$) after apelin-13 injection injection in the presence of benzamil hydrochloride, (mean± SD) was (106.5± 5.3 gram/second) compared to (90.6± 5 gram/second) before its injection.

In group III: there was a significance (P<0.001) increase the rate of development of tension ($+dT_{max}/t_{max}$) after apelin-13 injection in the presence of benzamil hydrochloride, (mean± SD) was (118.6± 9.5 gram/second) compared to (109.6± 8.4 gram/second) before its injection.

Furthermore, benzamil hydrochloride injection partially blocked the action of apelin as evidenced by the significant decrease in the percentage of increase in ($+dT_{max}/t_{max}$) in comparison with that produced by apelin alone in all groups (p<0.001).

Table 4 and record 5: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac relaxation parameter; rate of relaxation ($-dT_{max}/t_r$) [gram/second] in the presence of benzamil hydrochloride injection (10 nmol/kg) in the three main groups.

In group I: there was a significant (P<0.01) increase in the rate of relaxation (-dTmax/tr) after apelin-13 injection in the presence of benzamil hydrochloride, (mean± SD) was (41± 1.4 gram/second) compared to (37.3± 0.9 gram/second) before apelin-13 injection.

In group II: there was a significant (P<0.001) increase in the rate of relaxation (-dTmax/tr) after apelin-13 injection in the presence of benzamil hydrochloride, (mean± SD) was (35.8± 2.9 gram/second) compared to (30.1±1.9 gram/second) before its injection.

In group III: there was a significant (P<0.001) increase in the rate of relaxation (-dTmax/tr) after apelin-13 injection in the presence of benzamil hydrochloride, (mean± SD) was (41.2± 4.2 gram/second) compared to (37.4± 3.7 gram/second) before its injection. Furthermore, benzamil hydrochloride injection partially blocked the action of apelin as evidenced by thesignificant decrease in the percentage of increase in (-dTmax/tr) in comparison with that produced by apelin alone in all groups (P<0.001).

Table 5: Shows the effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on HR (beat\ min) in the three main groups.

In group I: there was a non-significant (P>0.05) change in HR after apelin-13 injection, (mean± SD) was (360± 19 beat\ mim) compared to (355± 22.6 beat\ mim) before its injection.

In group II: there was a non-significant (P>0.05) change in HR after apelin-13 injection, (mean± SD) was (325±12.2 beat\ mim) compared to (320± 15.5 beat\ mim) before its injection.

In group III: there was a non-significant (P>0.05) change in HR after apelin-13 injection, (mean± SD) was (355± 35.1 beat\ mim) compared to (350± 31 beat\ min) before its injection.

Table 1: Shows blood glucose levels (mg/dl) at the end of the studied period in all groups.

	Control	Diabetic	Diabetic treated
$\bar{X}$	78.1	413.5	81.5
SD	6.32	85.49	5.88
P value of LSD vs control	P<0.001		NS
P value of LSD vs diabetic		P<0.001	

NS: non-significant

Table (2): Shows the rate of development of tension ($+dT_{max}/t_{max}$)[gram/second]and rate of relaxation ($-dT_{max}/t_r$)[gram/second], and heart rate (beat/min) in all groups:

	$(+dT_{max}/t_{max})$ [gram/second]			**$(-dT_{max}/t_r)$** [gram/second]			**HR (beat/min.)**		
	Control	Diabetic	Diabetic treated	Control	Diabetic	Diabetic treated	Control	diabetic	Diabetic treated
$\bar{X}$	110.3	91.7	104.7	36.1	31.2	36.8	355	320	350
SD	12.8	5.2	10.7	2.3	3.6	0.8	22.6	15.5	31
P value of LSD vs control	P< 0.01		NS	<0.05		NS	<0.05		NS
Vs diabetic			P< 0.05			P<0.01			<0.05

NS: non-significant

Table (3): Shows the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) alone or in the presence of verapamil (4.8mg/kg), propranolol (0.2mg/kg) or benzamil Hcl (10 nmol/kg) on the rate of development of tension ($+dT_{max}/t_{max}$) [gram/second] in the three main groups.

		Control	**Diabetic**	**Diabetic Treated**
Apelin	*Before*	110.3±12.8	91.7±5.2	104.7±10.7
	After	133.3±12.9***	120.5±5.8***	127.5±10***
	% of increase	21.2±3.1	31.5±2.6***$	20.2±3***¥
Verapamil	*verapamil*	79±7.7	64.2±9.3	72.6±7.1
	verapamil + Apelin	94.3±9.3***	83.8±13***	85.3±9.6**
	% of increase	19.4±1.7€	27.5±2.6€	21.1±3.4€
Propranolol	*Propranolol.*	76.3±6.5	52±3.7	72.3±6.4
	Propranolol + Apelin	91.5±6.5***	66.6±4.5***	87.7±7.2**
	% of increase	19.9±2.5€	28±2.4€	21.4±2.4€
Benzamil Hcl	*Benzamil*	111.8±12.8	90.6±5	109.6±8.4
	Benzamil +Apelin	120.3±13.1**	106.5±5.3***	118.6±9.5***
	% of increase	7.5±2.4***€	17.7±2.2***€	8.2±1.9***€

** **Significant VS. pre-injection values of apelin P< 0.01**
*** **Significant VS. pre-injection values of apelin P< 0.001**
$ **VS control**
¥ **VS diabetic.**
€ **VS % of increase with Apelin alone**

Table (4): The effect of I.V. bolus injection of apelin-13 (10 nmol/kg) alone or in the presence of verapamil (4.8mg/kg) ,propranolol (0.2mg/kg) or benzamil hydrochloride (10 nmol/kg) on the rate of relaxation ($-dT_{max}/ t_r$) [gram/second] in the three main groups

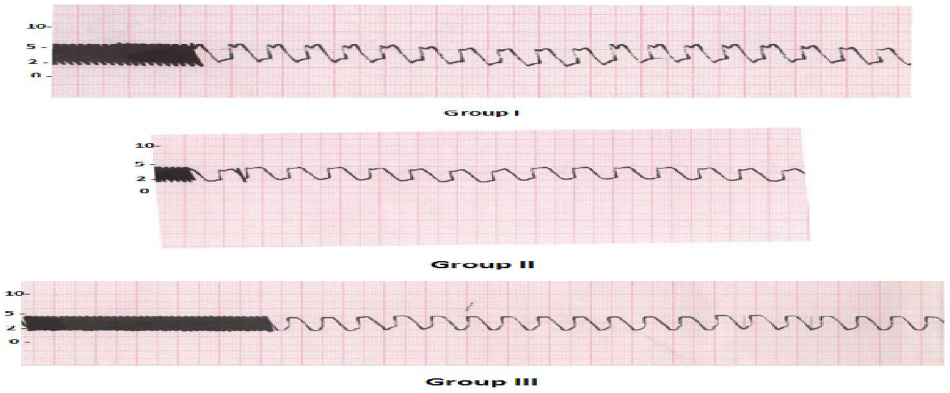
		Control	Diabetic	Diabetic Treated
Apelin	*Before*	36.1±2.3	30.8±2.8	37±1
	After	44.8±3.1***	40.5±2.9***	46.1±1.2***
	% of increase	23.1±4.1	29.8±3.2**$	25.5±3*¥
Verapamil	*Verapamil*	29.8±1.4	27.4±2.2	30.7±1.9
	verapamil + Apelin	37.2±2.5***	35.5±2.9***	38±2.5***
	% of increase	23.3±1.4€	29.8±1.5€	23.2±2.8€
Propranolol	*Propranolol.*	31.7±1.8	28.2±0.7	30.2±1.9
	Propranolol + Apelin	38.5±2.4***	36.7±1.3***	37.8±2.2***
	% of increase	22.8±2.8€	30.8±2.5€	25±3.1€
Benzamil Hcl	*Benzamil*	37.3±0.9	30.1±1.9	37.4±3.7
	Benzamil +Apelin	41±1.4**	35.8±2.9***	41.2±4.2***
	% of increase	9.7±2.6***€	19±4.3***€	9.7±2.6***€

** **Significant VS. pre-injection values of apelin P< 0.01**
*** **Significant VS. pre-injection values of apelin P< 0.001**
$ **VS control**
¥ **VS diabetic .**
€ **VS % of increase with Apelin alone**

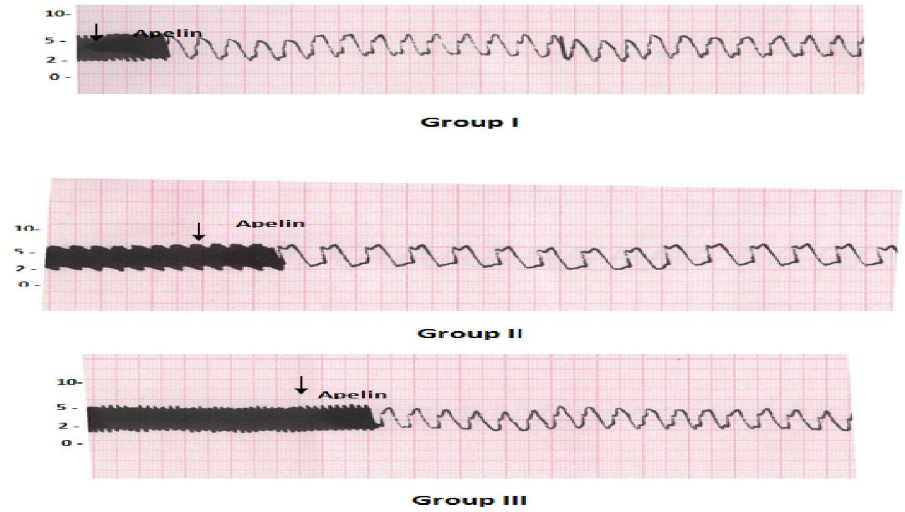
Table (5): The effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on HR (beat\ min) in the three main groups.

		Control	Diabetic	Diabetic Treated
Apelin	Before	355±22.6	320±15.5	350±31
	After	360±19	325±12.2	355±35.1
P value of paired t test		NS	NS	NS

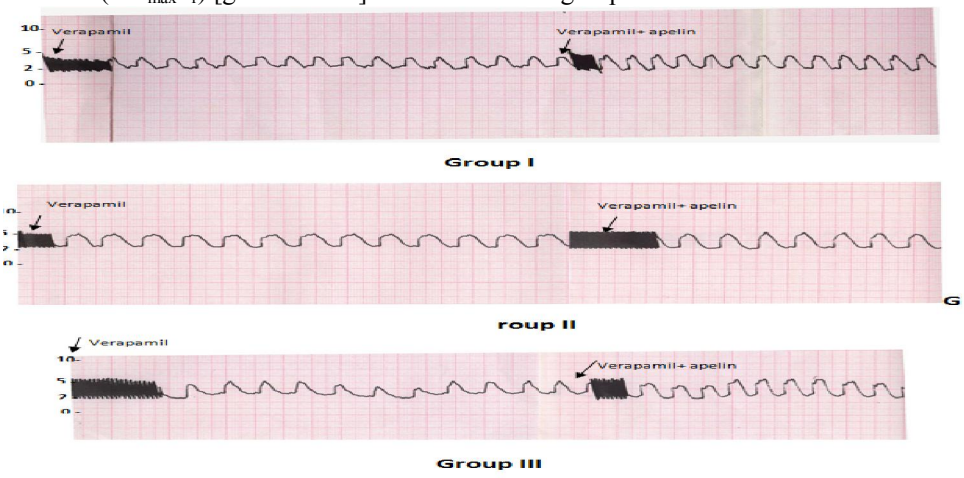
NS:non-significant



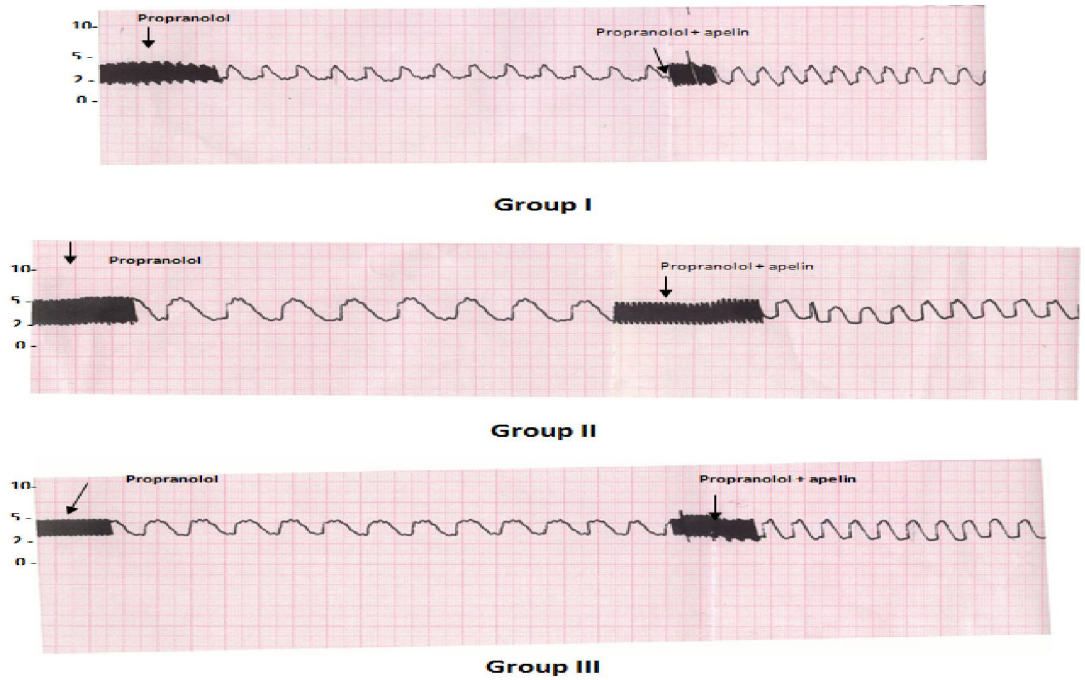
Record 1: Shows (+ dT_{max}/t_{max}) [gram/second] and (-dT_{max}/t_r) [gram/second] in the three main groups.



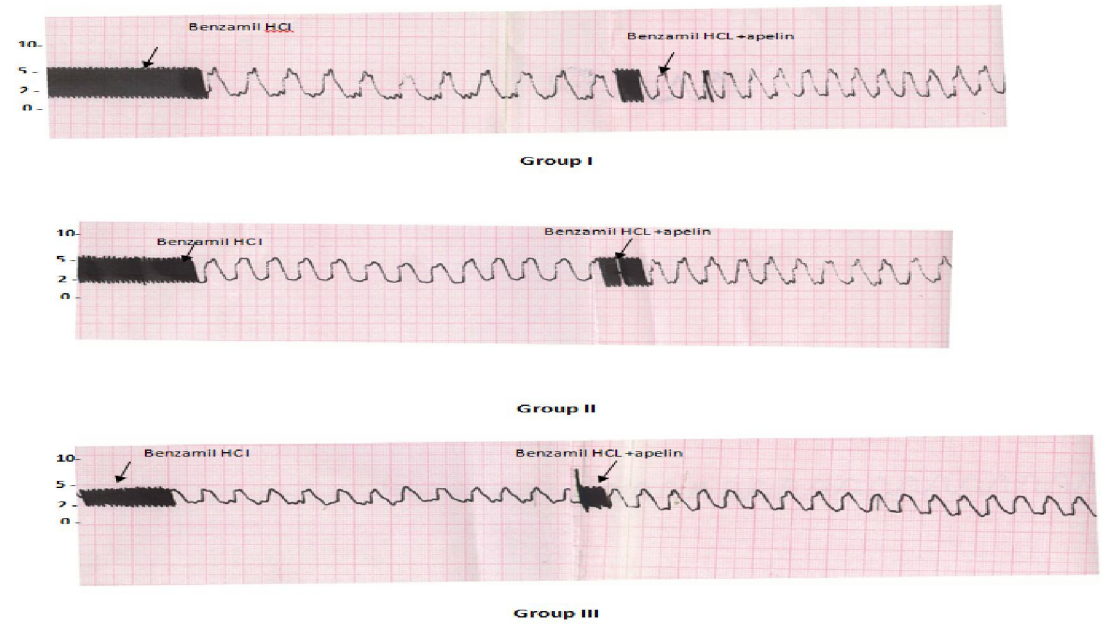
Record 2: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac contractility parameter; the rate of development of tension (+dT_{max}/t_{max}) [gram/second] and cardiac relaxation parameter; rate of relaxation (-dT_{max}/t_r) [gram/second] in the three main groups.



Record 3: Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac contractility parameter; the rate of development of tension (+dTmax/tmax) [gram/second] and cardiac relaxation parameter; rate of relaxation (-dTmax/tr) [gram/second] in the presence of verapamil injection (4.8 mg/kg) in the three main groups.



Record 3: Shows the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on cardiac contractility parameter; the rate of development of tension ($+dT_{max}/t_{max}$) [gram/second] and on cardiac relaxation parameter; rate of relaxation ($-dT_{max}/t_r$) [gram/second] in the presence of propranolol injection (0.2mg/kg) in the three main groups.



Record 4: Shows the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) the rate of development of tension ($+dT_{max}/t_{max}$) [gram/second] and the rate of relaxation ($-dT_{max}/t_r$) [gram/second] in the presence of benzamil hydrochloride injection (10 nmol/kg) in the three main groups.

4. Discussion

Apelin is the endogenous ligand for the previously orphaned G-protein–coupled receptor, APJ. This novel pathway is widely expressed in the cardiovascular system and is emerging as an important mediator of cardiovascular homeostasis **(Japp *et al.*, 2010).**

In our study, streptozotocin induced diabetic rats had a significant weight loss (about 25%) and displayed typical manifestations of diabetes mellitus such as polydypsia, polyurea, and hyperglycemia. The results of this study showed that both contraction and relaxation of cardiac muscle were significantly reduced in case of diabetic rats as indicated by the significant decrease in ($+dT_{max}/t_{max}$) and ($-dT_{max}/t_r$) in comparison with that of both control and insulin treated groups. In addition, there was a significant decrease in heart rate in the diabetic rats as compared with that of the two other groups of rats.

Our results are in agreement with those of who concluded that STZ-diabetic rats exhibited a significant decrease in indices of both contractility and relaxation as compared to control rats and STZ-diabetic rats treated with insulin **(Borges *et al.*, 2006).**

This can be explained as follows; in STZ diabetic rats the ability of the sarcoplasmic reticulum to take up and release calcium is depressed. Similarly reports for decreases in Na^+/K^+ATPase and adenylyl cyclase accompanied by decreases in sodium/calcium exchanges and calcium pump activity have been documented in diabetes **(Nordin and Gilat, 1990)**. In addition to cardiomyopathy, alteration in the lipid metabolism seems to be another factor involved in cardiac depression **(Shenoy and Goyal, 2002).**

Furthermore, myocardial dysfunction is an important feature that might be associated with a number of intrinsic alterations of cardiac myocytes **(Ren and Bode, 2000)**. There are several studies *in vivo* (anesthetized animals) and *in vitro* (Langhendorff and isolated myocytes) showing an impairment of Ca^{++} homeostasis and Ca^{++}signaling in diabetes **(Ren *et al.*, 2000, and Choi *et al.*, 2002)**.The most significant abnormalities involved delay of the relaxation process, slow relaxation ratio and delay in peak ratio of isometric and isotonic relaxation **(Choi *et al.*, 2002)**.

Finally, an impairment of sympathetic innervations of the heart, frequently observed in diabetes **(Maeda *et al.*, 1995 and Fazan *et al.*, 1999)**, should be also taken into consideration in the impairment of myocardial contractility found in STZ-diabetic rats.

Moreover, the development of STZ-induced bradycardia has been attributed to a down regulation of myocardial beta adrenoceptors **(Baba and Ishikawa, 1992)**, and depression of myocardial calcium metabolism **(Nordin and Gilat, 1990)**.

In addition, in the present study, treatment with insulin prevented the occurrence of alterations caused by diabetes, i.e. bradycardia and low ($+dT_{max}/t_{max}$) and ($-dT_{max}/t_r$).

Although several studies demonstrated that insulin can prevent, or even reverse, the derangements caused by chronic diabetes (**Fein *et al.*, 1981, Schaan *et al.*, 1997**). Nevertheless, the mechanism responsible for this protective effect is still unknown, because diabetes is a long-standing metabolic disorder with several outcomes. It has been demonstrated in normal cardiac myocytes that insulin speeds the glucose transport into the cell **(Bayliss *et al.*, 1928)**. However, it has been demonstrated also that insulin promotes a positive inotropic effect independent of glucose uptake **(Oye and Sinclair, 1966)**.

Our results are in line with those of **Stroedter *et al.* (1995)** who reported the improvement of cardiac performance in diabetes following the subcutaneous administration of insulin. They also suggested that the dysfunction of the heart observed in diabetes may be caused by conspicuous alterations of myocardial metabolism caused by insulin deficiency, which can be reversed by means of exogenous replacement of the hormone.

Moreover, the results of this work demonstrated that apelin-13 administration significantly increased cardiac muscle performance without any significant changes in heart rate , in all groups, as evidenced by the significant increase in ($+dT_{max}/t_{max}$) and ($-dT_{max}/t_r$). In addition, this increase was more significant in diabetic rats in comparison with that of both control and diabetic treated rats

Our results are supported by those of other investigators who concluded that acute apelin infusion increases cardiac contractility and cardiac output **(Berry *et al.*, 2004, Jia *et al.*, 2006, Atluri *et al.*, 2007)**, furthermore, other studies reported significant increase in the diastolic function of the heart after apelin injection **(Berry *et al.*, 2004, , Pan *et al.*, 2010).**

The mechanisms by which apelin exerts its inotropic effects have been only partially elucidated and remain the subject of debate. However, in our study the observed effects are independent of ATP calcium channels or B- adrenergic receptors but appear to involve activation of the sacrolemmal Na^{++}/Ca^{++} exchanger (NCX), as verapamil failed to attenuate the inotropic response to apelin. Moreover, the effect of apelin remained unchanged in the presence of propranolol .On the other hand, administration of benzamil HCL (Na/Ca^{+2} exchange (NCX) blocker) partially blocked the effect of apelin-13 injection on the cardiac performance.

Our results are in line with **Dai *et al.* (2006)** who reported that in intact rat hearts, inhibition of NCX suppresses the apelin-induced inotropic response indicating that this mechanism may contribute to apelin-mediated inotropic activity, they

also concluded that apelin increased the amplitude of the intracellular $Ca2^{+}$ transient **(Dai *et al.*, 2006).** Moreover, **Kentish, 1999** concluded that, apelin does not alter voltage-gated Ca^{++} channels in cardiomyocytes.

In addition, the positive inotropic effect of apelin is independent of angiotensin II, endothelin-1, catecholamines and nitric oxide release **(Szokodi *et al.*, 2002)** but appear to involve activation of the sacrolemmal Na^{+}/H+ exchanger (NHE), probably through phospholipase C and protein kinase C-dependent pathways **(Szokodi *et al.*, 2002, Farkasfalvi *et al.*, 2007)**. In single cardiomyocytes, NHE activity increases following exposure to apelin while, in intact rat hearts, the inotropic response to apelin is markedly attenuated by a specific inhibitor of NHE. Stimulation of NHE can lead to intracellular alkalinization and sensitization of cardiac myofilaments to intracellular Ca^{++} **(Karmazyn *et al.*, 1999)**. In keeping with this, the increased NHE activity is accompanied by an increase in intracellular pH **(Farkasfalvi *et al.*, 2007)**. Moreover, activation of NHE can also indirectly increase intracellular Ca^{++} as the resulting accumulation of Na^{+} within cells stimulates the reverse mode Na^{+}/Ca^{++} exchanger (NCX) **(Karmazyn *et al.*, 1999, Kentish *et al.*, 1999).**

Thus the inotropic effects of apelin may involve increased intracellular Ca^{++} availability in addition to enhanced myofilament responsiveness to Ca^{++} ions **(Japp and Newby, 2008).**

The results of the above studies suggest that activation of NHE and NCX contributes to the inotropic effect of apelin, whereas voltage-activated Ca2are not involved, whatever, the finding that 40% of the apelin-induced positive inotropic effect remained unaffected even after combined inhibition of NHE and NCX indicates the existence of additional signaling mechanisms **(Berry *et al.*, 2004).**

Furthermore, the effect of apelin on myocardial efficiency could be mediated also via PKC **(Ashley *et al.*, 2005)** this is because cardiac apelin–APJ signaling is abrogated by PKC inhibitors and PKC phosphorylation of the cardiac fibers has been shown to reduce the requirements of the contractile apparatus for both calcium and ATP (promoting efficient ATP utilization) **(Pi *et al.*, 2003)**. Furthermore, apelin injection increased coronary blood flow to the cardiac muscle **(Japp *et al.*, 2010).**

In addition, our results are supported by the following studies who reported that apelin has positive inotropic effects in vivo in both normal rat hearts and rat hearts in failure after myocardial infarction (**Szokodi *et al.*, 2002, Berry *et al.*, 2004, Dai *et al.*, 2006),** and so apelin may have used as an acute inotropic agent in patients with ischemic heart failure **(Berry *et al.*, 2004)**. Interestingly, an apelin-knockout mice showed severely impaired heart contractility **(Kuba *et al.*, 2007)**, which suggests that decrease in endogenous apelin plays a pivotal role in heart failure **(Berry *et al.*, 2004, Atluri *et al.*, 2007; Sheikh *et al.*, 2008).**

Lastly, the findings of the more significant effects in diabetic rats in comparison with that of both control and insulin treated rats, might be explained, at least partially, by means of an up-regulation of APJ receptors exhibited by STZ-diabetic rats, which may be due to decrease in apelin synthesis and secretion in the injured endothelium and myocardium **(Jia *et al.*, 2006)**, even though this hypothesis deserves better investigation.

In addition to the above explanation, **Dray *et al.* (2008)** demonstrated that acute injection of apelin was able to improve glucose tolerance and to increase glucose utilization in heart; this noticeable effect needs to be further depicted.

However, in addition to confirming the *in vivo* positive inotropic effect, **Ladeiras-Lopes *et al.* (2008)** demonstrated that apelin has a negative inotropic effect in isolated cardiac muscle, suggesting other cells may be required in addition to myocardial cells so that positive inotropic effect is revealed.

As regards the effect of apelin-13 on the heart rate, our results are in agreement with those of **Lee *et al.* (2000)**, who reported insignificant changes in heart rate after apelin injection. While those results are in disagreement with the results of other investigators who concluded that apelin injection decreased heart rate in rodents **(Tatemoto *et al.*, 1998).**

Moreover, our finding also in controversy to those of other investigators who reported that IV apelin injection increased heart rate in conscious sheep and both anaesthetized and conscious rats **(Cheng *et al.*, 2003, Charles *et al.*, 2006)**.

The reason for these discrepancies among findings is unclear; however, possible explanations are as follows: in case of anaesthetized rats, anesthetics are well known to affect the sympathetic nervous system **(Kagiyama *et al.*, 2005)**. Moreover, the diversity of the previous results may be due to differences in methodology and the different doses of apelin administered **(Chamdrasekaran *et al.*, 2008).** In addition, cardiovascular response to apelin may exhibit interspecies differences **(Japp and Newby *et al.*, 2008)**

Conclusion,

Apelin-13 exerted both positive inotropic and lusiotropic effects without affection of the heart rate in vivo, which was more significant in diabetic rats in comparison with that of both normal and insulin

treated rats. Our results also suggested that this response to apelin involved activation of Na^{+}- Ca^{+2} exchange channels (NCX).

Since different mechanisms are responsible for the diabetic cardiomyopathy and response rate to treatments is far from homogenous and ideal, the search for additional therapeutic agents continues. Therefore, the use of apelin may be investigated as a potential therapeutic target for this pathology. Furthermore, studies examining the effects of chronic apelin administration on long-term cardiac function will also be useful in assessing apelin treatment of chronic heart failure

Finally, more studies are recommended to investigate not yet discovered mechanism/s of apelin actions on the cardiovascular system on the cardiac performance.

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References

1. Ashley EA, Powers J, Chen M, Kundu R, Finsterbach T, Caffarelli A, Deng A, Eichhorn J, Mahajan R, Agrawal R, Greve J, Robbins R, Patterson AJ, Bernstein D, Quertermous T (2005): The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. Cardiovasc Res., 65:73–82.
2. Atluri P, Morine KJ. Liao GP, Panlilio CM, Berry MF, Hsu VM Hiesinger W, Cohen JE, Joseph Woo Y (2007): Ischemic heart failure enhances endogenous myocardial apelin and APJ receptor expression, Cell. Mol. Biol. Lett. 12: 127–138.
3. Avogaro A, Vigili de Kreutzenberg S, Negut C, Tiengo A, Scognamiglio R (2004): Diabetic cardiomyopathy: a metabolic perspective. The American Journal of Cardiology 93 (8A), 13A–16A.
4. Bayliss LE, Muller EA, Starling EH (1928): The action of insulin and sugar on the respiratory quotient and metabolism of the heartlung preparation. J Physiol, 65:33-47.
5. Berry MF, Pirolli TJ, Jayasankar V Burdick J, Morine KJ, Gardner TJ, and Woo YJ (2004): Apelin has in vivo inotropic effects on normal and failing hearts. Circulation, 110: II187–II193
6. Borges GR, Oliveira M,Salgado HC, Jr RF (2006): Myocardial performance in conscious streptozotocin diabetic rats. Cardiovascular Diabetology, 5:26
7. Boucher J, Masri B, Daviaud D, Gesta S, Guigné C, Mazzucotelli A, Castan- Laurell I, Tack I, Knibiehler B, Carpéné C, Audigier Y, Saulnier-Blache J S, Valet P (2005): Apelin, a newly identified adipokine up-regulated by insulin and 877 obesity. Endocrinology 146: 1764–1771.
8. Chandersekaran B, Dar O, McDonagh (2008): The role of apelin in cardiovascular function and heart failure, European journal of heart failure 10: 725-732.
9. Chen MM, Ashley EA, Deng DX, Tsalenko A, Deng A, Tabibiazar R, Ben- Dor A, Fenster B, Yang E, King JY, Fowler M, Robbins R, Johnson FL, Bruhn L, McDonagh T, Dargie H, Yakhini Z, Tsao PS, Quertermous T (2003): Novel role for the potent endogenous inotrope apelin in human cardiac dysfunction. Circulation 108:1432–1439
10. Cheng X, Cheng XS, Pang CC (2003): Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats. Eur J Pharmacol, 470:171–5.
11. Choi KM, Zhong Y, Hoit BD,Grupp IL, Hahn H, Dilly KW, Guatimosim S, Lederer WJ, Matlib MA (2002): Defective intracellular Ca (2+) signaling contributes to cardiomyopathy in Type 1 diabetic rats. Am J Physiol Heart Circ Physiol, 283:H1398-H1408.
12. Dai T, Ramirez-Correa G, Gao WD (2006): Apelin increases contractility in failing cardiac muscle. Eur J Pharmacol, 553:222–228.
13. Dray C, Knauf C, Daviaud D, Wage A, Boucher J, Bule´ on M, Cani PD, Attane´C , Guigne´C , Carpe´ ne´C , Burcelin R, Castan-Laurell I, Valet P (2008): Apelin Stimulates Glucose Utilization in Normal and Obese Insulin-Resistant Mice Cell Metabolism, 8: 437–445.
14. Farkasfalvi K, Stagg MA, Coppen SR, Siedlecka U, Lee J, Soppa GK, Marczin N, Szokodi I, Yacoub MH, Terracciano CM (2007): Direct effects of apelin on cardiomyocyte contractility and electrophysiology. Biochem Biophys Res Commun .357:889–895.
15. Fazan R Jr., Dias da Silva VJ, Ballejo G, Salgado HC (1999): Power spectra of arterial pressure and heart rate in streptozotocin-induced diabetes in rats. J Hypertens, 17:489-495.
16. Gay W I (1965): Methods of animal experimentation.Vols 1 - 7 Academic Pess, New York. USA.
17. Gosh MN (1971): Fundamental of experimental pharmacology. 1st edition, Scientific Book Agency, Calcutta.
18. Goyal RK, Lakkad NB, Rao MV (1997): Effects of chronic treatment with enalapril on streptozotocin-induced diabetic rats. Pharmacol Rev Comm.5:201-209.
19. Heinonen M V, Purhonen AK, Miettinen P, Paakkonen M, Pirinen E, Alhava E, Akerman K, Herzig K H (2005): Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. Regul. Pept. 130: 7–13.
20. Japp AG and Newby DE (2008): The apelin-APJ system in heart failure: pathophysiologic relevance and therapeutic potential. Biochem Pharmacol .75:1882–1892..
21. Japp AG, Cruden N L, Barnes G, van Gemeren N, Mathews J, Adamson J, Johnston N R, Denvir M A, Megson I L, Flapan A D, Newby D E (2010): Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure, Circulation;121;1818-1827.
22. Kadoglou NP, Lampropoulos S, Kapelouzou A, Gkontopoulos A, Theofilogiannakos EK, Fotiadis G, Kottas G (2010): Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease—kozani study. Transl Res. 155: 238 246.
23. Kagiyama S, Fukuhara M, Matsumura K, Lin Y, Fujii K, Iida M (2005): Central and peripheral cardiovascular actions of apelin in conscious rats. Regul Pept ; 125:55–9
24. Karmazyn M, Gan XT, Humphreys RA, Yoshida H, Kusumoto K (1999): The myocardial Na (+)–H (+) exchange: structure, regulation, and its role in heart disease. Circ Res, 85:777–86.
25. Kentish JC (1999): A role for the sarcolemmal Na (+)/H (+) exchanger in the slow force response to myocardial stretch. Circ Res, 85:658–60.
26. Kuba K, Zhang L, Imai Y, Arab S, Chen M, Maekawa Y, Leschnik M, Leibbrandt A, Markovic M, Schwaighofer J, Beetz N, Musialek R, Neely GG, Komnenovic V, Kolm U, Metzler B, Ricci R, Hara H, Meixner A, Nghiem M, Chen X, Dawood F, Wong KM, Sarao R, Cukerman E, Kimura A, Hein L, Thalhammer J, Liu PP, Penninger JM (2007): Impaired heart contractility in Apelin gene-deficient mice associated with aging and pressure overload. Circ Res. 101(4):e32-42.
27. Ladeiras-Lopes R, Ferreira-Martins J, Leite-Moreira AF (2008): The apelinergic system:the role played in human

physiology and pathology and potential therapeutic applications. Arq Bras Cardiol; 90:343–9.
28. Lee D K, Cheng R, Nguyen T, Fan T, Kariyawasam A P, Liu Y, Osmond D H, George S R, O'Dowd B F (2000): Characterization of apelin, the ligand for the APJ receptor. J. Neurochem. 74: 34–41.
29. Li Z, Bai Y, Hu J (2008*):* Reduced Apelin Levels in Stable Angina. Inter Med 47: 1951-1955.
30. Lutz A.J. and Pardridge W.M. (1993): Insulin therapy normalizes glucose transporter mRNA but not immunoreactive transporter protein in streptozotocin-diabetic rats. Metab. 42: 939-944.
31. Macaluso NJ, Pitkin SL, Maguire JJ, Davenport AP, Glen RC. (2011) :Discovery of a competitive apelin receptor (APJ) antagonist. ChemMedChem. Jun 6;6(6):1017-23.
32. Maeda CY, Fernandes TG, Timm HB, Irigoyen MC (1995): Autonomic dysfunction in short-term experimental diabetes. Hypertension, 26:1100-1104.
33. Nishimura M, Ohtsuka K, Nanbu A, Takahashi H, and Yoshimura M (1998): Benzamil blockade of brain Na^+ channels averts Na^+-induced hypertension in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R635-R644.
34. Nordin C and Gilat E (1990): Delayed after depolarisation and activity in ventricular muscle from rats in STZ induced diabetes. Circ Res, 57: 28-34.
35. Oye I and Sinclair D (1966): Insulin in myocardial infarction. Lancet, 1:366.
36. Pan C, Teng X, Zhang, Cai Y, Zhao J, Wu W, Wang X, Tang C, and Qi Y (2010): Apelin antagonizes myocardial impairment in sepsis. J Cardiac Fail 16: 609-617.
37. Persson KG, Burger AP, Mugabo P, Green I (2007): In vivo effects of Crinum macowanii on the Rat Cardiovascular System. MSc Thesis ,Department of Medical Bioscience, University of the Western Cape.
38. Pi Y, Zhang D, Kemnitz KR, Wang H, Walker JW (2003): Protein kinase C and A sites on troponin I regulate myofilament Ca2+ sensitivity and ATPase activity in the mouse myocardium. J Physiol; 552: 845– 57
39. Przewlocka-Kosmala M, Kotwica T, Mysiak A, Kosmala W (2011) Reduced circulating apelin in essential hypertension and its association with cardiac dysfunction. J Hypertens. May; 29(5):971-9.
40. Rastaldo R, Cappello S, Folino A, Berta GN, Sprio AE, Losano G, Samaja M, Pagliaro P.(2011) : Apelin-13 limits infarct size and improves cardiac postischemic mechanical recovery only if given after ischemia. Am J Physiol Heart Circ Physiol. Jun;300(6):H2308-15
41. Ren J and Bode AM (2000): Altered cardiac excitation-contraction coupling in ventricular myocytes from spontaneously diabetic BB rats. Am J Physiol Heart Circ Physiol. 279: H238-H244.
42. Ronkainen VP, Ronkainen JJ, Hanninen SL, Leskinen H, Ruas JL, Pereira T, Poellinger L, Vuolteenaho, O., Tavi P (2007): Hypoxia inducible factor regulates the cardiac expression and secretion of apelin. Faseb J. 21: 1821-1830.
43. Schaan BD, Maeda CY, Timm HB, Medeiros S, Moraes RS, Ferlin E, Fernandes TG, Ribeiro JP, Schmid H, Irigoyen MC (1997): Time course of changes in heart rate and blood pressure variability in streptozotocin-induced diabetic rats treated with insulin. Braz JMed Biol Res, 30:1081-1086.
44. Sheikh AY, Chun HJ, Glassford AJ, Kundu RK, Kutschka I, Ardigo D, Hendry SL,Wagner RA, Chen MM, Ali ZA, et al. (2008): In vivo genetic profiling and cellular localization of apelin reveals a hypoxia-sensitive, endothelial-centered pathway activated in ischemic heart failure. *Am J Physiol Heart Circ Physiol* 294:H88–H98.
45. Shenoy A G and Goyal R K (2002): Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocin-diabetic rats. Indian Journal of Pharmacolog,y 34: 156-164..
46. Sivitz WI, Walsh S, Morgan D, Donohoue P, Haynes W, Leibel L (1998): Plasma leptin in diabetic and insulin treated diabetic and normal rats. Metab. 47: 584-591.
47. Srinivasan P S, Hakim Z S,Santani D and K. Goyal R K (1997): Effects of chronic treatment with amlodipine in streptozotocindiabetic and spontaneously hypertensive rats. Department of Pharmacology, L.M. College of Pharmacy, Navrangpura, Ahmedabad-380 009, India
48. Stroedter D, Schmidt T, Bretzel RG, Federlin K (1995): Glucose metabolism and left ventricular dysfunction are normalized by insulin and islet transplantation in mild diabetes in the rat. Acta Diabetol, 32:235-243..
49. Szokodi I, Tavi P, Foldes G, Voutilainen-Myllyla S, Ilves M, Tokola H, Pikkarainen S, Piuhola J, Rysa J, Toth M, Ruskoaho H (2002): Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ Res 91:434–440.
50. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou M, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H, Fujino M (1998): Isolation and characterization of a novel endogenous peptide ligand for the human apj receptor . Biochemical and biophysical research communications: 251: 471–476.
51. Vongpatanasin W, Mansour Y, Chavoshan B, Arbique D, Victor GR. (1999): Cocaine Stimulates the Human Cardiovascular System via a Central Mechanism of Action. Circulation, 100: 497-502
52. Wei L, Hou X, Tatemoto K (2005): Regulation of apelin mRNA expression by insulin and glucocorticoids in mouse 3T3-L1 adipocytes Regul. Pept. 132: 27-32.
53. Weir RA, Chong KS, Dalzell JR, Petrie CJ, Murphy CA, Steedman T, Mark PB, McDonagh TA, Dargie HJ, McMurray JJ (2009): Plasma apelin concentration is depressed following acute myocardial infarction in man. Eur J Heart Fail. 11:551–558.
54. Yves MH and Theo FM(2007): The effect of low dose insulin on mechanical sensitivity and allodynia in type1 diabetes neuropathy. Neurosc. Letters, 417: 149-154.
55. Zeng XX, Wilm TP, Sepich DS, Solnica-Krezel L (2007): Apelin and its receptor control heart field formation during zebrafish gastrulation. Dev Cell,12: 391–402

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