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 (+d$T_{max}$/ $t_{max}$) and (-d$T_{max}$/t). 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$^{2+}$ 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.

[Aim: Apelin expression in adipose tissue is regulated by nutritional status, such as fasting and refeeding (Boucher et al., 2005). 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 3 main 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 streptozotocin-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 ml of sodium citrate, at pH 4.5 (Lutz and Partridge, 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 streptozotocin-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 intraperitoneally 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 (10 nmol/kg) on cardiac muscle performance 10 minutes after the propranolol injection (0.2 mg/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
10 minutes after the verapamil (ca \(^{2+}\) channel blocker) injection (4.8 mg/kg) (Persson et al., 2007) in the three main groups (n=18).

**Experiment IV:** to study the acute effect of apelin-13 injection (10 nmol/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 statistically investigated in all experiments (this effect was about 5-10 minutes after its injection).

### Calculation of the studied parameters

1- **Maximum tension developed (+dT\(_{\text{max}}\))**: It was obtained from the calibration of tension on the graph in grams.

2- **Time to reach maximum tension (t\(_{\text{max}}\))**: 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 1 mm measured on the baseline equal to 0.02 sec. According to the latter equation the time to reach maximum tension (t\(_{\text{max}}\)) was calculated in seconds.

3- **Rate of developing tension (+dT\(_{\text{max}}\)/t\(_{\text{max}}\))**: By dividing Maximum tension developed (+dT\(_{\text{max}}\)) by time to reach maximum tension (t\(_{\text{max}}\)) 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 1 mm equals 0.02 sec.

5- **Rate of cardiac relaxation (-dT\(_{\text{max}}\)/t\(_{r}\))**: By dividing the maximum tension developed by the time of cardiac relaxation (t\(_{r}\)) the rate of cardiac relaxation (-dT\(_{\text{max}}\)/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 ± 85.49 mg/dl) was significantly increased (P< 0.001) when compared with that of group I (78.1 ± 6.32 mg/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 ± 5.88 mg/dl, P < 0.001).

**Table 2 and record 1:** Show cardiac contractility parameter; the rate of development of tension (+dT\(_{\text{max}}\)/t\(_{\text{max}}\)) [gram/second] and cardiac relaxation parameter; rate of relaxation (-dT\(_{\text{max}}\)/t\(_{r}\)) [gram/second] and heart rate in the three main groups. There was a significant decrease in (+dT\(_{\text{max}}\)/t\(_{\text{max}}\))[gram/second] (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 (-dT\(_{\text{max}}\)/t\(_{r}\)) [gram/second]: (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 (+dT\(_{\text{max}}\)/t\(_{\text{max}}\)) [gram/second] in the three main groups.

In group I: there was a significant (P< 0.001) increase in (+dT\(_{\text{max}}\)/t\(_{\text{max}}\)) (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 (+dT\(_{\text{max}}\)/t\(_{\text{max}}\)) (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 (+dT\(_{\text{max}}\)/t\(_{\text{max}}\)) (78.1± 6.32 gram/second) to (104.8± 10.7 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 (+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 (+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 4: 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 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 (-dTmax/tr) 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 II: there was a significant (P<0.001) increase in the rate of relaxation (-dTmax/tr) 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 (-dTmax/tr) 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 (+dTmax/tmax) [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 (+dTmax/tmax) 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 (+dTmax/tmax) 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 (+dTmax/tmax) 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 (+dTmax/tmax) 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 (-dTmax/tr) [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 (-dTmax/tr) after apelin-13 injection in the presence of propranolol, (mean± SD) was (38.5± 2.9 gram/second) compared to (29.8± 1.4 gram/second) before apelin-13 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 at a dose of 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 (-dT_{max}/t_r) 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 (-dT_{max}/t_r) 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 (-dT_{max}/t_r) 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 the significant decrease in the percentage of increase in (-dT_{max}/t_r) 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\ mim) before its injection.

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (X)</td>
<td>78.1</td>
<td>413.5</td>
<td>81.5</td>
</tr>
<tr>
<td>Standard Deviation (SD)</td>
<td>6.32</td>
<td>85.49</td>
<td>5.88</td>
</tr>
<tr>
<td>P value of LSD vs control</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value of LSD vs diabetic</td>
<td></td>
<td>P&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th></th>
<th>(+dT(<em>{max}/t</em>{max})) [gram/second]</th>
<th>(-dT(_{max}/t_r)) [gram/second]</th>
<th>HR (beat/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
<td>Diabetic treated</td>
</tr>
<tr>
<td>X</td>
<td>110.3</td>
<td>91.7</td>
<td>104.7</td>
</tr>
<tr>
<td>SD</td>
<td>12.8</td>
<td>5.2</td>
<td>10.7</td>
</tr>
<tr>
<td>P value of LSD vs control</td>
<td>P&lt; 0.01</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apelin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>133.3±12.9***</td>
<td>120.5±5.8***</td>
<td>127.5±10***</td>
<td></td>
</tr>
<tr>
<td>% of increase</td>
<td>21.2±3.1</td>
<td>31.5±2.6***$</td>
<td>20.2±3***$</td>
<td></td>
</tr>
<tr>
<td>verapamil</td>
<td>79±7.7</td>
<td>64.2±9.3</td>
<td>72.6±7.1</td>
<td></td>
</tr>
<tr>
<td>Verapamil + Apelin</td>
<td>94.3±9.3***</td>
<td>83.8±13***</td>
<td>85.3±9.6**</td>
<td></td>
</tr>
<tr>
<td>% of increase</td>
<td>19.4±1.7$</td>
<td>27.5±2.6$</td>
<td>21.1±3.4$</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>76.3±6.5</td>
<td>52±3.7</td>
<td>72.3±6.4</td>
<td></td>
</tr>
<tr>
<td>Propranolol + Apelin</td>
<td>91.5±6.5***</td>
<td>66.6±4.5***</td>
<td>87.7±7.2**</td>
<td></td>
</tr>
<tr>
<td>% of increase</td>
<td>19.9±2.5$</td>
<td>28±2.4$</td>
<td>21.4±2.4$</td>
<td></td>
</tr>
<tr>
<td>Benzamil Hcl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzamil + Apelin</td>
<td>120.3±13.1**</td>
<td>106.5±5.3***</td>
<td>118.6±9.5***</td>
<td></td>
</tr>
<tr>
<td>% of increase</td>
<td>7.5±2.4***$</td>
<td>17.7±2.2***$</td>
<td>8.2±1.9***$</td>
<td></td>
</tr>
</tbody>
</table>

** 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.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apelin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>36.1±2.3</td>
<td>30.8±2.8</td>
<td>37±1</td>
</tr>
<tr>
<td>After</td>
<td>44.8±3.1***</td>
<td>40.5±2.9***</td>
<td>46.1±1.2***</td>
</tr>
<tr>
<td>% of increase</td>
<td>23.1±4.1</td>
<td>29.8±3.2**</td>
<td>25.5±3*</td>
</tr>
<tr>
<td><strong>Verapamil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verapamil + Apelin</td>
<td>37.2±2.5***</td>
<td>35.5±2.9***</td>
<td>38±2.5***</td>
</tr>
<tr>
<td>% of increase</td>
<td>23.3±1.4€</td>
<td>29.8±1.5€</td>
<td>23.2±2.8€</td>
</tr>
<tr>
<td><strong>Propranolol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propranolol + Apelin</td>
<td>38.5±2.4***</td>
<td>36.7±1.3***</td>
<td>37.8±2.2***</td>
</tr>
<tr>
<td>% of increase</td>
<td>22.8±2.8€</td>
<td>30.8±2.5€</td>
<td>25±3.1€</td>
</tr>
<tr>
<td><strong>Benzamil Hcl</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzamil + Apelin</td>
<td>41±1.4**</td>
<td>35.8±2.9***</td>
<td>41.2±4.2***</td>
</tr>
<tr>
<td>% of increase</td>
<td>9.7±2.6***€</td>
<td>19±4.3***€</td>
<td>9.7±2.6***€</td>
</tr>
</tbody>
</table>

*** Significant VS. pre-injection values of apelin P< 0.001
** Significant VS. pre-injection values of apelin P< 0.01
s VS control
v 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.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apelin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>355±22.6</td>
<td>320±15.5</td>
<td>350±31</td>
</tr>
<tr>
<td>After</td>
<td>360±19</td>
<td>325±12.2</td>
<td>355±35.1</td>
</tr>
<tr>
<td>P value of paired t test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: non-significant
Record 1: Shows \((+ \frac{dT_{\text{max}}}{t_{\text{max}}})\) [gram/second] and \((-\frac{dT_{\text{max}}}{t_{r}})\) [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 \((+\frac{dT_{\text{max}}}{t_{\text{max}}})\) [gram/second] and cardiac relaxation parameter; rate of relaxation \((-\frac{dT_{\text{max}}}{t_{r}})\) [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 \((+\frac{dT_{\text{max}}}{t_{\text{max}}})\) [gram/second] and cardiac relaxation parameter; rate of relaxation \((-\frac{dT_{\text{max}}}{t_{r}})\) [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\textsubscript{max}/t\textsubscript{max}) [gram/second] and on cardiac relaxation parameter; rate of relaxation (-dT\textsubscript{max}/t\textsubscript{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\textsubscript{max}/t\textsubscript{max}) [gram/second] and the rate of relaxation (-dT\textsubscript{max}/t\textsubscript{t}) [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) 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 adenyl 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 (Langendorff 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 (Baha 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).}

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). 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 sarcoplemmal 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++ exchange 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 Ca$^+$ 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 sarcolemmal 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 alkalization 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 Ca$^{2+}$are not involved, however, 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 (Kagiyma et al., 2005). Moreover, the diversity of the previous results may be due to differences in methodology and the different doses of apelin administered (Chandrakeshwar 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⁺-Ca²⁺ 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
37. Persson KG, Burger AP, Mugah 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.