

## Effect of Acute Apelin Injection on Arterial Blood Pressure 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. The next studies have demonstrated that apelin and its receptor are involved in the regulation of cardiovascular function. It has been hypothesized that it may exert vasodilating and hypotensive effects as opposed to the pressor action of angiotensin II/ angiotensin-type 1 receptor (AT1) signaling. 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 arterial blood pressure in both normal and diabetic state, with a trial to clarify possible involved mechanisms. **Material & methods:** This study was conducted on 90 healthy adult male albino rats; the animals were divided equally into 3 equal main groups: **Group I:** Control group, **Group II:** Strptozotocin -induced type 1 diabetes non-treated rats, and **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) either alone or in the presence of, L-NAME (10 mg/kg b.wt) or Glibenclamide (20 mg/kg) on mean arterial pressure (MAP) and heart rate (HR), also we investigated the effect of acute apelin injection on MAP reactivity to angiotensin II (60 ng/kg b.wt) or acetylcholine (1 µg/kg). **Results:** The results of this study showed that administration of apelin-13 in normal, diabetic, and diabetic treated anaesthetized rats was reduced MAP by 10, 19.3, 10.2 %, respectively ( $p < 0.001$ ), without any significant change in heart rate. In the presence of a nitric oxide (NO) synthase inhibitor (L-Name), the effect of apelin-13 on blood pressure was abolished. However, the administration of apelin-13 in the presence of glibenclamide produced a significant decrease in MAP in all groups. Thus, apelin may lower blood pressure via a nitric oxide-dependent mechanism. The results of this work showed also that, administration of apelin-13 antagonizes the hypertensive action of Ang II in all groups, furthermore, this effect was less significant in the diabetic rats in comparison with that of control and insulin treated groups. In addition, our results demonstrated that administration of apelin-13 produced a significant increase in the hypotensive effect of acetylcholine in all groups, moreover, this effect of apelin-13 was more significant in diabetic group in comparison with that of both control and diabetic treated groups. **Conclusion:** acute apelin-13 administration *in vivo* caused NO-mediated decrease in mean arterial blood pressure, which was more significant in diabetic rats in comparison with normal and insulin treated rats. In addition, apelin-13 injection antagonized the hypertensive action of Ang II which was less significant in the diabetic group, and augmented the hypotensive effect of acetylcholine which was more significant in the diabetic rats in comparison with that of control and insulin treated groups. Therefore, the use of apelin may be investigated as a potential therapeutic target for diabetic vasculopathy. However, the impact of chronic administration requires further attention.

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**Key words:** Apelin, diabetes, blood pressure, rats

### 1. Introduction

Apelin is a 36 amino-acid peptide identified in 1998 as the endogenous ligand of the orphan G protein-coupled APJ receptor, (Tatemoto *et al.*, 2001 and Japp & Newby, 2008). This peptide is a multiple functional peptide that regulates body fluid homeostasis, food intake, respiratory rhythm and biological rhythm (Taheri *et al.*, 2002), as apelin and APJ mRNA are widely expressed in several rat and human tissues and have functional effects in both the central nervous system and periphery as heart, lung, gastrointestinal tract, adipose tissue, adrenal gland,

and endothelium (Jia *et al.*, 2006 and Mazzucotelli *et al.*, 2008).

The apelin receptors are localized in vascular smooth muscle of the human coronary artery, aorta and saphenous vein (Kleinz & Davenport, 2004), the endothelium of the small arteries (Jia *et al.*, 2006) as well as in rat and human myocardia (Kleinz & Davenport, 2004 and Macaluso *et al.*, 2011). Furthermore, the administration of apelin causes a significant decrease in mean arterial blood pressure (Cheng *et al.*, 2003, Ishida *et al.*, 2004, Chun *et al.*, 2008), on the other hand, there are also reports of apelin's vasopressor response in human vascular

system (Katugampola *et al.*, 2001 and Kagiya *et al.*, 2005)

Recently it was reported that in patients with essential hypertension, circulating apelin levels are reduced, and lower plasma apelin could be associated with left ventricular systolic and diastolic function impairment (Przewlocka- Kosmala *et al.*, 2011)

Taken together, it can be concluded that, apelin has an important regulatory role in cardiovascular homeostasis (Masri *et al.*, 2005). However, this role and the significance of apelin in cardiovascular diseases remain to be clarified (Japp & Newby, 2008).

Diabetes mellitus is one of the major risk factors for cardiovascular disease which is the leading cause of death in those patients (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).

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

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

## 2. Animals and Methods

### Animals:

This study was conducted on 90 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 mean arterial blood pressure (MAP) and heart rate of normal rats.

**Group II:** To study the acute effect of apelin-13 injection (10 nmol /kg) (Cheng *et al.*, 2003) on MAP and heart rate of streptozotocin - induced type I 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) The rats were provided with oral 10% glucose solution after 6 hours of streptozotocin administration for the next 48 hours.

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). And the rats maintained for 6 weeks (Srinivasan *et al.*, 1997, Shenoy and Goyal, 2002).

**Group III:** To study the acute effect of apelin-13 injection (10 nmol /kg b.wt) on MAP and heart rate of streptozotocin -induced type I 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 arterial blood pressure and electrocardiogram (ECG)(via 4-channel oscillograph "MD4" (Bioscience , London):** The rats were anaesthetized with ethyl carbamate (urethane<sup>®</sup>) in a dose 1.75-2 gm /kg body weight injected intraperitoneally as 25 % freshly prepared aqueous solution (Gosh, 1971).

After stabilization of anesthesia, a midline longitudinal skin incision started just below the neck and extended to the sternum was done. 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 internal jugular vein was carefully exposed and cannulated for drug administration. The carotid artery was also exposed and cannulated with a polyethylene arterial cannula (with 3-way valve) filled with heparinized saline solution (16 I.U/ml), and was connected to PT 400 blood pressure transducer. The ECG limb cable was attached through hypodermic needles inserted and fixed subcutaneously, in axilla (for each forelimb), just above the ankle (for each hind limb) and to the chest above the apex of the heart. The FC123 was switched on lead II that gives good signal for the analysis of ECG, the selected paper chart speed for recording the ECG by the oscillograph was 25 mm/ sec., 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).

After stabilization of the blood pressure and heart rate for 30 minutes, experiment was performed.

#### Experimental design

**Experiment I:** to study the acute effect of apelin-13 (10 nmol/kg b.wt) (Cheng *et al.*, 2003) on MAP and heart rate in the three main groups (n=18).

**Experiment II:** to study the acute effect of apelin-13 (10 nmol/kg b.wt) (Cheng *et al.*, 2003) on MAP and heart rate 10 minutes after L-NAME (nitric oxide synthase inhibitor) injection (10 mg/kg b.wt) (Gardiner *et al.*, 1990) in the three main groups (n=18).

**Experiment III:** to study the acute effect of apelin-13 (10 nmol/kg) (Cheng *et al.*, 2003) on MAP and heart rate 10 minutes after Glibenclamide (a selective KATP channel inhibitor) injection, (20 mg/kg) (O'shaughnessy *et al.*, 1992) in all groups (n=18).

**Experiment IV:** to study the effect of apelin-13 injection (10 nmol/kg b.wt) on the MAP reactivity to angiotensin II (60 ng/kg) injection (Lee *et al.*, 2000) in the three main groups (n=18).

**Experiment V:** to study the acute effect of apelin-13 injection (10 nmol/kg b.wt) on the MAP reactivity to acetylcholine injection (1 µg/kg b.wt) (O'shaughnessy *et al.*, 1992) in the three main groups (n=18).

**NB:** The maximal effect of apelin injection on MAP and HR was calculated and statistically investigated in all experiments (this effect was about 5 minutes after its injection).

#### 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/l) 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 mean arterial blood pressure (MAP) and heart rate (beat/min) in the three main groups: There was a significant increase

in MAP (mean± SD) (111.1± 12.9 mm Hg) in diabetic group in comparison with that of both control (90.5± 7.6 mm Hg, P<0.01) and insulin treated (93.6± 6.6 mmHg, P<0.01) groups. 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 at a dose of 10 nmol/kg on MAP (mm Hg) in the three main groups.

In group I: there was a significant (P<0.001) decrease in mean arterial blood pressure after apelin-13 injection, (mean± SD) was (81.6±6.7 mmHg) compared to (90.5±7.6 mmHg) before its injection.

In group II: there was a significant (P<0.001) decrease in mean arterial blood pressure after apelin-13 injection, (mean± SD) was (90.2±12 mmHg) compared to (111.1±12.9 mmHg) before its injection.

In group III: there was a significant (P<0.001) decrease in mean arterial blood pressure after apelin-13 injection, (mean± SD) was (83.4±5.3 mmHg) compared to (93.6±6.6 mmHg) before its injection.

Moreover, the percentage of reduction was more significant in diabetic group (group II), (mean± SD) was (19.3±2.1) compared to that of both group I (10±1.2, P < 0.001) and group III (10.3±1, P < 0.001).

**Table 3 and record 3:** Show the effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on MAP (mmHg) in presence of L-NAME injection (10 mg/kg) in the three main groups.

In group I: there was a non-significant (P>0.05) change in MAP after apelin-13 injection in the presence of L- NAME, (mean ± SD) was (116.9± 5 mm Hg) compared to (117.8± 4.2 mm Hg) before apelin-13 injection.

In group II: there was a non-significant (P>0.05) change in MAP after apelin-13 injection in the presence of L- NAME, (mean± SD) was (162.2± 12.4 mm Hg) compared to (165.5±14.6 mm Hg) before its injection.

In group III: there was a non-significant (P>0.05) change in MAP after apelin-13 injection in the presence of L- NAME, (mean± SD) was (116.5±12.4 mm Hg) compared to (117.8± 11.7 mm Hg) before its injection.

**Table 3 and record 4:** Show the effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on MAP (mmHg) in presence of glibenclamide injection (20 mg/kg) in the three main groups.

In group I: there was a significant (P<0.001) decrease in MAP after apelin-13 injection, (mean ± SD) was (81.3± 11.8 mmHg) compared to (91.9 ± 10.2 mmHg) before its injection.

In group II: there was a significant ( $P < 0.001$ ) decrease in MAP after apelin-13 injection, (mean  $\pm$  SD) was ( $87.3 \pm 11.4$  mmHg) compared to ( $108.4 \pm 14.7$  mmHg) before its injection.

In group III: there was a significant ( $P < 0.001$ ) decrease in MAP after apelin-13 injection, (mean  $\pm$  SD) was ( $81.2 \pm 11.9$  mmHg) compared to ( $91.5 \pm 14.6$  mmHg) before its injection.

Furthermore, no significant difference was detected in the percentage of reduction in MAP in the presence of glibenclamide in comparison to that produced by apelin alone in all groups.

**Table 4 and record 2:** Show 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  $\pm$  SD) was ( $360 \pm 19$  beat\ min) compared to ( $355 \pm 22.6$  beat\ min) before its injection.

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

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

**Table 4 and record 3** Show the effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on HR (beat\ min) in presence of L-NAME injection (10 mg/kg) in the three main groups.

In group I: there was a non-significant ( $P > 0.05$ ) change in heart rate after apelin-13 injection in the presence of L- NAME, (mean  $\pm$  SD) was ( $355 \pm 22.6$  beat\ min) compared to ( $365 \pm 22.6$  beat\ min) before its injection.

In group II: there was a non-significant ( $P > 0.05$ ) change in heart rate after apelin-13 injection in the presence of L- NAME, (mean  $\pm$  SD) was ( $335 \pm 22.6$  beat\ min) compared to ( $330 \pm 26.8$  beat\ min) before its injection.

In group III: there was a non-significant ( $P > 0.05$ ) change in heart rate after apelin-13 injection in the presence of L- NAME, (mean  $\pm$  SD) was ( $365 \pm 12.4$  beat\ min) compared to ( $370 \pm 24.5$  beat\ min) before its injection.

**Table 4 and record 4:** Show the effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on HR (beat\ min) in presence of glibenclamide injection (20 mg/kg) in the three main groups.

In group I: there was a non-significant ( $P > 0.05$ ) change in heart rate after apelin-13 injection in the presence of glibenclamide, (mean  $\pm$  SD) was ( $350 \pm 15.5$  beat\ min) compared to ( $355 \pm 22.6$  beat\ min) before its injection

In group II: there was a non-significant ( $P > 0.05$ ) change in heart rate after apelin-13 injection in the presence of glibenclamide, was (mean  $\pm$  SD) ( $335 \pm 22.6$  beat\ min) compared to ( $330 \pm 26.8$  beat\ min) before its injection

In group III: there was a non-significant ( $P > 0.05$ ) change in heart rate after apelin-13 injection in the presence of glibenclamide, (mean  $\pm$  SD) was ( $365 \pm 22.6$  beat\ min) compared to ( $370 \pm 24.5$  beat\ min) before its injection

**Table 5 and record 5:** Show the effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on MAP reactivity to angiotensin II injection (60 ng/kg) in the three main groups.

In group I: the hypertensive effect of angiotensin II was completely blocked by the combined administration of apelin-13, as MAP was significantly ( $P < 0.001$ ) reduced from (mean  $\pm$  SD) ( $119.1 \pm 11.5$  mm Hg) to ( $95.2 \pm 8.8$  mm Hg).

In group II: the hypertensive effect of angiotensin II was partially blocked by the combined administration of apelin-13, as MAP was significantly ( $P < 0.001$ ) reduced from (mean  $\pm$  SD) ( $165.5 \pm 23.3$  mm Hg) to ( $149.4 \pm 22.2$  mm Hg).

In group III: the hypertensive effect of angiotensin II was completely blocked by the combined administration of apelin-13, as MAP was significantly ( $P < 0.001$ ) reduced from (mean  $\pm$  SD) ( $125.2 \pm 6.2$  mm Hg) to ( $100.2 \pm 4.8$  mm Hg). Moreover, the percentage of decrease in MAP was less significant in diabetic group (group II), (mean  $\pm$  SD) was ( $9.7 \pm 2.2$ ) compared to that of group I ( $20.3 \pm 2.7$ ,  $p < 0.01$ ) and group III ( $20.2 \pm 1.7$ ,  $p < 0.01$ ).

**Table 5 and record 6:** Show the effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on the MAP reactivity to acetylcholine injection (1  $\mu$ g/kg) in the three main groups.

In group I: the hypotensive effect of acetylcholine was significantly ( $P < 0.01$ ) increased from (mean  $\pm$  SD) ( $61.7 \pm 3.8$  mmHg) to ( $55.9 \pm 5.6$  mmHg) by combined apelin injection.

In group II: the hypotensive effect of acetylcholine was significantly ( $P < 0.001$ ) increased from (mean  $\pm$  SD) ( $77.6 \pm 3.9$  mmHg) to ( $60.5 \pm 2.7$  mmHg) by combined apelin injection.

In group III: the hypotensive effect of acetylcholine was significantly ( $P < 0.001$ ) increased from (mean  $\pm$  SD) ( $62.4 \pm 2.7$  mmHg) to ( $58.3 \pm 2.1$  mmHg) by combined apelin injection.

Moreover, the percentage of decrease in MAP caused by the combined injection of acetylcholine and apelin was more significant in diabetic group (group II), (mean  $\pm$  SD) ( $21.8 \pm 5.2$ ) compared to that of group I ( $9.2 \pm 5.1$ ,  $P < 0.001$ ) and group III ( $6.7 \pm 1.9$ ,  $P < 0.001$ ).

**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
$\pm$ SD	6.32	85.49	5.88
<b>P value of LSD</b>	<0.001		NS
		<0.001	

NS: non-significant

**Table (2): Shows MAP (mmHg) and heart rate (beat\ min) in the three main groups.**

	MAP in all groups			HR in all groups		
	Control	Diabetic	Diabetic treated	Control	Diabetic	Diabetic treated
$\bar{X}$	90.5	111.1	93.6	355	320	350
$\pm$ SD	7.6	12.9	6.6	22.6	15.5	31
<b>P value of LSD</b>	< 0.01		NS	<0.05		NS
		< 0.01			<0.05	

NS: non-significant

**Table (3): The effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on MAP (mmHg) alone or in the presence of L-NAME injection (10 mg/kg), glibenclamide injection (20 mg/kg) in the three main groups.**

		Control	Diabetic	Diabetic Treated
<b>Apelin</b>	Before	90.5 $\pm$ 7.6	111.1 $\pm$ ....	93.6 $\pm$ 6.6
	After	81.6 $\pm$ 6.7 <sup>***</sup>	90.2 $\pm$ 12 <sup>***</sup>	83.4 $\pm$ 5.3 <sup>***</sup>
	% of reduction	10 $\pm$ 1.2	19.3 $\pm$ 2.1 <sup>***S</sup>	10.3 $\pm$ 1 <sup>***Y</sup>
<b>L-NAME</b>	L-NAME	117.8 $\pm$ 4.2	165.5 $\pm$ 14.6	117.8 $\pm$ 11.7
	L-name+ Apelin	116.9 $\pm$ 5	162.2 $\pm$ 12.4	116.5 $\pm$ 12.4
<b>Glibenclamide</b>	Glib.	91.9 $\pm$ 10.2	108.4 $\pm$ 14.7	91.5 $\pm$ 14.6
	Glib + Apelin	81.3 $\pm$ 11.8 <sup>***</sup>	87.3 $\pm$ 11.4 <sup>***</sup>	81.2 $\pm$ 11.9 <sup>***</sup>
	% of reduction	11.7 $\pm$ 3.1 <sup>€</sup>	18.9 $\pm$ 3 <sup>€</sup>	10.8 $\pm$ 2 <sup>€</sup>

\*\* Significant VS. pre-injection values P&lt; 0.01

\*\*\* Significant VS. pre-injection values P&lt; 0.001

<sup>S</sup> VS control <sup>Y</sup> VS diabetic . <sup>€</sup> VS % of reduction with Apelin alone**Table (4): The effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on HR (beat/min) alone or in the presence of L-NAME injection (10 mg/kg) , glibenclamide injection (20 mg/kg) in the three main groups (Mean  $\pm$ SD)**

		Control	Diabetic	Diabetic Treated
<b>Apelin</b>	Before	355 $\pm$ 22.6	320 $\pm$ 15.5	350 $\pm$ 31
	After	360 $\pm$ 19	325 $\pm$ 12.2	355 $\pm$ 35.1
<b>L-NAME</b>	L-NAME	365 $\pm$ 22.6	330 $\pm$ 26.8	370 $\pm$ 24.5
	L-name+ Apelin	355 $\pm$ 22.6	335 $\pm$ 22.6	365 $\pm$ 12.4
<b>Glibenclamide</b>	Glib.	355 $\pm$ 22.6	330 $\pm$ 26.8	370 $\pm$ 24.5
	Glib + Apelin	350 $\pm$ 15.5	335 $\pm$ 22.6	365 $\pm$ 22.6



**Table (5): The effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on MAP (mmHg) reactivity to Angiotensin II injection (60 ng/kg) or Acetyl Choline injection (1 µg/kg) in the three main groups.**

		Control	Diabetic	Diabetic Treated
<b>Angiotensin.II</b>	Basal Level	91.6±6.8	122.7±13.7	94.2±6.8
	Ang. II	119.1±11.5** <sup>a</sup>	165.5±23.3** <sup>a</sup>	125.2±6.2*** <sup>a</sup>
	Ang.II+ Apelin	95.2±8.8*** <sup>b</sup>	149.4±22.2*** <sup>b</sup>	100.2±4.8*** <sup>b</sup>
	%of reduction	20.3±2.7	9.7±2.2** <sup>s</sup>	20.2±1.7** <sup>¥</sup>
<b>Acetyl Choline</b>	Basal Level	90.5±7.7	122.8±11.2	93.6±7.5
	ACH	61.7±3.8*** <sup>a</sup>	77.6±3.9** <sup>a</sup>	62.4±2.7*** <sup>a</sup>
	ACH+ Apelin	55.9±5.6** <sup>c</sup>	60.5±2.7*** <sup>c</sup>	58.3±2.1*** <sup>c</sup>
	%of reduction	9.2±5.1	21.8±5.2*** <sup>s</sup>	6.7±1.9*** <sup>¥</sup>

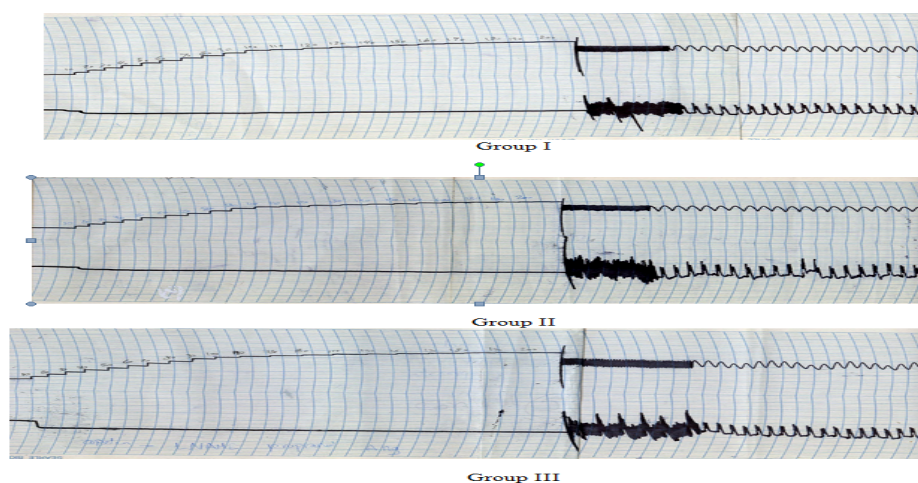
a-Significant vs. baseline b-Significant vs. Ang.II c- Significant vs. ACH

\*\* Significant (P< 0.01)

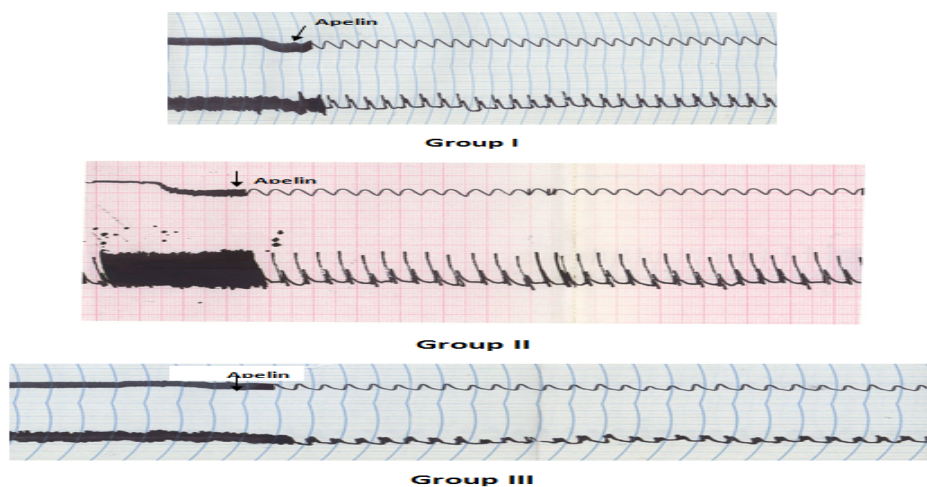
\*\*\* Significant (P< 0.001)

<sup>s</sup> VS control

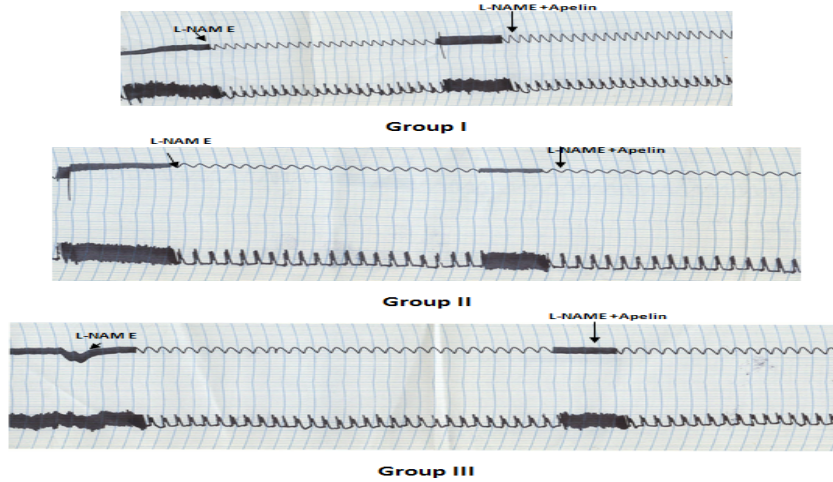
<sup>¥</sup> VS diabetic .



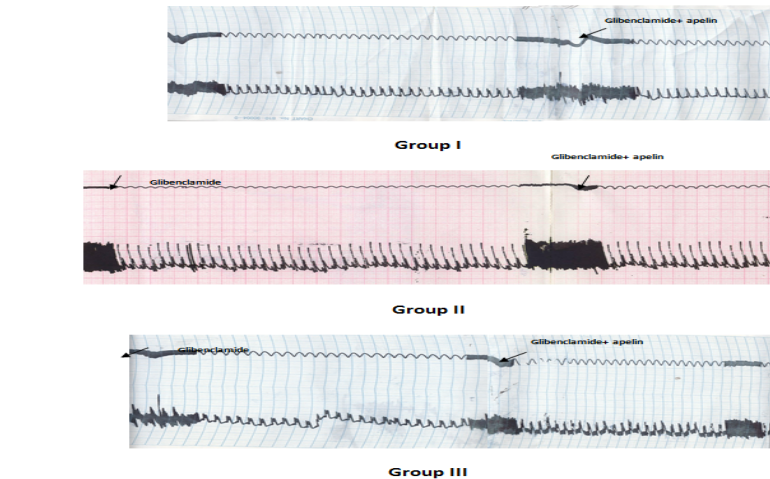
**Record 1: Shows arterial blood pressure and heart rate in all groups.**



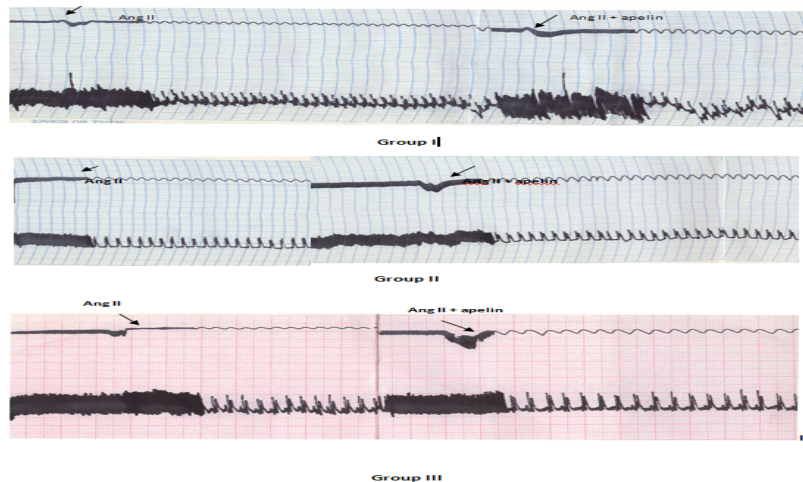
**Record 2: Effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on arterial blood pressure (mmHg) and HR (beat\ min) in the three main groups.**



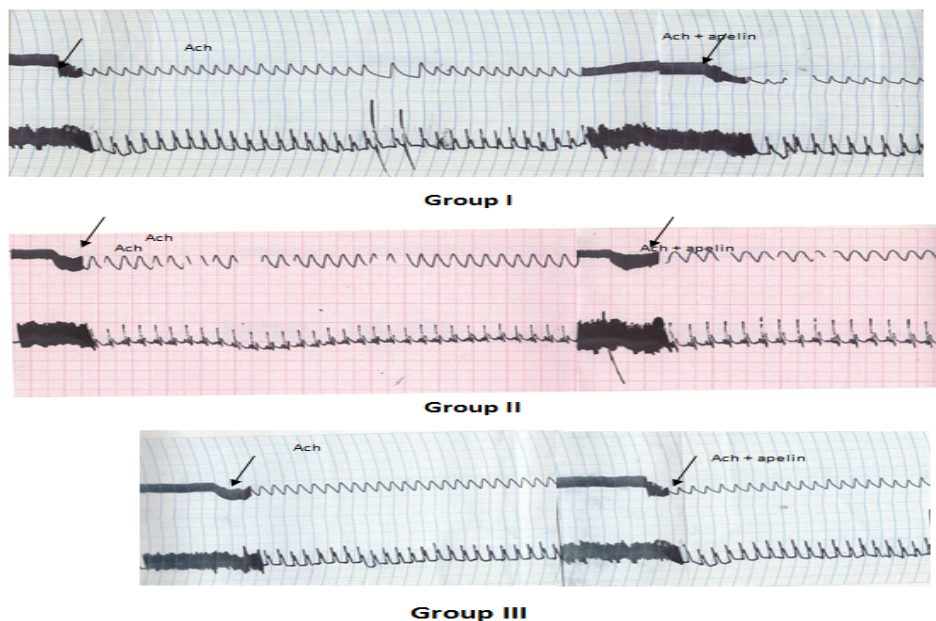
**Record 3: The effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg in on arterial blood pressure (mmHg) and HR (beat\ min) in the presence of L-NAME 10 mg/kg in the three main groups.**



**Record 4: Effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg following injection of glibenclamide (20 mg/kg) on arterial blood pressure (mmHg) and HR (beat\ min) in the three main groups.**



**Record 5: The effect of I.V. bolus injection of apelin-13 at a dose of 10 nmol/kg on arterial blood pressure reactivity to angiotensin II injection (60 ng/kg) in the three main groups.**



**Record 6: The effect of I.V. bolus injection of apelin-13 (10 nmol/kg) on arterial blood pressure reactivity to acetylcholine injection (1  $\mu$ g/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.*, 2008).

In our study, streptozotocin induced diabetic rats had a significant weight loss (about 25%) and displayed typical manifestations of diabetes mellitus such as polydipsia, polyurea, and hyperglycemia. Our results showed a significant increase in the mean arterial blood pressure and a significant decrease in heart rate in diabetic rats in comparison with that of both normal and insulin treated groups.

Such an increase in blood pressure has also been reported by other researchers (Rodrigues *et al.*, 1986), moreover, bradycardia has been frequently observed in STZ diabetic rats (Shah *et al.*, 1995, Sevak and Goyal, 1996, Goyal *et al.*, 1997, Borges *et al.*, 2006).

A number of factors are involved in the pathogenesis of hypertension in diabetes mellitus such as sodium retention, extracellular fluid volume expansion, altered activity of sympathetic nervous system and renin-angiotensin system (RAS), and increased vascular reactivity towards noradrenaline and angiotensin-I (Srinivasan *et al.*, 1997).

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).

Furthermore, the results of this study showed that MAP after the administration of apelin-13 at a dose of 10 nmol/kg in normal, diabetic, and diabetic treated anaesthetized rats was reduced by 10, 19.3, and 10.2 %, respectively. In the presence of a nitric oxide (NO) synthase inhibitor (L- Name), the effect of apelin-13 on blood pressure was abolished. However, the administration of apelin-13 in the presence of glibenclamide produced a significant decrease in MAP in all groups. Thus, apelin may lower blood pressure via a nitric oxide-dependent mechanism.

In agreement with our results, intravenous (IV) injection of apelin decreased blood pressure in rodents, and this response was inhibited by pretreatment with nitric oxide synthase inhibitor (Tatemoto *et al.*, 2001 and Mitra *et al.*, 2006). Moreover, Cheng *et al.* (2003) reported that the intravenous injection of apelin decreased arterial pressure in conscious rats. Furthermore, Lee *et al.* (2005) concluded that apelin can lower blood pressure in normotensive rats and to a much greater extent in the hypertensive rats. They also explained that the similar decreases in both systolic and diastolic blood pressure were indicative of an arterial vasodilatation effect.

Our in vivo findings are in line with those of Salcedo *et al.*, 2007 who demonstrated that NO synthase, but not cyclooxygenase, inhibition attenuated relaxation to apelin in human mesenteric



arteries in vitro. **Japp et al., 2008** also concluded that administration of apelin in vivo in man causes NO-mediated arterial vasodilatation but does not appear to affect peripheral venous tone. However, a venous vasodilator effect of apelin was reported in conscious rats (**Cheng et al., 2003**).

The mRNA transcripts of the apelin receptor APJ were detected in the endothelium of blood vessels. This suggests that apelin may stimulate the NO production via activation of eNOS. Thus, apelin may be a new member of the family of vasoactive substances that includes bradykinin, acetylcholine, and serotonin, a group of agents that are known to activate eNOS in the endothelial cells (**Tatemoto et al., 2001**).

Accordingly, the hypotensive effect of apelin is mediated by endothelium-derived NO, since the NO synthase inhibitor L-NAME abolished this effect both in rats (**Tatemoto et al., 2001**) and in mice (**Ishida et al., 2004**). In addition, apelin increases plasma concentration of NO metabolites, nitrites and nitrates (**Negri, 2007**). Moreover, in cultured mice endothelial cells, apelin stimulates the phosphorylation of endothelial NO synthase (eNOS) by protein kinase B/Akt. This pathway plays an important role in the regulation of eNOS activity; it is activated, for example, by several other vasodilators, such as insulin and leptin (**Cheng et al., 2003, Ishida et al., 2004, Lee et al., 2005**).

In addition, blood pressure regulation by apelin also appears to be modulated by the CNS (**Lee et al., 2006**). As APJ receptor is widely distributed in the central nervous system, especially in the hypothalamic paraventricular nucleus (PVN) and the supraoptic nucleus, which are the central terminals for cardiovascular regulation (**O'Carroll et al., 2003**).

In contrast to our study, **Reaux et al. (2001)** reported that Intracerebroventricular (ICV) injection of apelin did not change arterial pressure in anesthetized rats. Moreover, apelin mutant mice did not show an apparent increase in blood pressure (**Kuba et al., 2007**).

However, **Kagiyama et al. (2005)** study demonstrated that central and peripheral injection of apelin-13 increased MAP and HR in conscious rats, they also reported that this pressor response to apelin was related to the direct effect of apelin on peripheral sympathetic nervous system.

Whatever, in conscious sheep low doses of apelin induced no significant alterations in arterial pressure and at a higher dose a clear biphasic arterial pressure response was observed consisting of initial hypotension followed by hypertension (**Charles et al., 2006**). This was accompanied by reciprocal heart

rate changes that were most likely baroreflex mediated (**Charles et al., 2006**).

Lastly, **Katugampola et al. (2001)** reported a vasoconstrictor effect of apelin-13 on denuded human saphenous vein with potency similar to endothelin-1. Thus, apelin can act directly on APJ receptors within vascular smooth muscle to induce contraction but this effect is outweighed by stimulation of local nitric oxide production via endothelial APJ receptors (**Ishida et al., 2004**).

As regard the effect of apelin-13 on the heart rate, our results are in agreement with those of **Tatemoto et al. (2001)**, 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 insignificant change in heart rate in spite of the significant decrease in MAP after apelin injection in our study may be due to the inhibitory effect of anesthesia on the baroreceptor reflex, as urethane anesthesia is well known to attenuate the baroreceptor reflex by about four to five-folds (**Fluckiger et al., 1985**).

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**).

The results of this work showed also that, administration of apelin-13 completely blocked the hypertensive action of Ang II in both of normal and diabetic treated groups but this block was partial in the diabetic group.

Our results are in agreement with those of **Zhong et al. (2005)** who reported that apelin-APJ system revealed a counter-regulatory role against the Ang II-AT1 signaling, leading to subsequent reduction of NO inactivation in the spontaneously hypertensive rat (**Zhong et al., 2004 and Zhong et al., 2005**).

This can be explained as follows, apelin may exert vasorelaxation effect via activation of eNOS pathway (**Tatemoto et al., 2001 and Ashley et al.,**

2006). In addition, apelin may phosphorylate serine/threonine kinase Akt and this will activate eNOS phosphorylation pathway and promote NO release (Ishida *et al.*, 2004, Kleinz and Davenport, 2004, Cox *et al.*, 2006, Masri *et al.*, 2006, , Grisk, 2007, Zhong *et al.*, 2007). In contrast, Ang II can produce reactive oxygen species (ROS) and impair phosphatidyl inositol 3-kinase (PI3K)-dependent activation of Akt/eNOS phosphorylation, which in turn diminishes NO generation, causing potent vasoconstriction (Endemann and Schiffrin, 2004 and Kim *et al.*, 2006).

As abnormalities in the Akt-eNOS phosphorylation pathway would result in reduction of NO production and impairment of vascular reactivity (Li *et al.*, 2005, Kim *et al.*, 2006, Masri *et al.*, 2006), therefore, apelin may play a crucial role in the vascular tone maintenance by counteracting the vasoconstrictor action of Ang II and potentiating the release of NO through activation of Akt-eNOS phosphorylation pathway, and so, improved endothelium-dependent relaxation in diabetic rats (Zhong *et al.*, 2007).

Interestingly, APJ-deficient mice showed an increased vasopressor response to angiotensin II, and the baseline blood pressure of double mutant mice homozygous for both APJ and angiotensin-type IA receptor was significantly elevated compared with that of angiotensin type I receptor-deficient mice (Ishida *et al.*, 2004). These results demonstrate that apelin/APJ plays a counter-regulatory role against the pressor action of angiotensin II. Moreover, this inhibitory activity is likely mediated by increased endothelial NO production and blocking Ang II signaling to the nucleus, with some portion of the latter mediated by physical association of AT1R and APJ receptors (Chun *et al.*, 2008).

Lastly, the partial block produced by apelin in case of diabetic rats versus the complete block in both normal and diabetic treated groups may be explained by the exaggerated contractile response of the vascular smooth muscle to angiotensin II (Carpéné *et al.*, 2007).

In addition, our results demonstrated that administration of apelin-13 produced a significant increase in the hypotensive effect of acetylcholine in all groups, moreover, this effect of apelin-13 was more significant in diabetic group in comparison with that of both control and diabetic treated groups.

Those results are in line with those of Zhong *et al.* (2007) study who described that a marked decrease in dose-dependent vasorelaxation response to acetylcholine was shown in aortic rings and renal arteries from diabetic mice, implying reduced bioavailability of NO under basal conditions in diabetes. More intriguingly, pretreatment with apelin

strikingly improved the vasorelaxation responses to acetylcholine, which was abolished by L-NAME in aortic arteries from diabetic mice, indicating that apelin may potentially improve endothelial function to some extent via eNOS phosphorylation pathway in diabetes. Subsequently, SNP (a direct NO donor) supplement restored a normal degree of the vascular smooth muscle vasodilatory response, indicating endothelial rather than smooth muscle dysfunction in diabetes.

The finding of the more significant effect of apelin-13 injection 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 (Atluri *et al.*, 2007); as apelin expression could have been less in diabetic than in control vascular tissue, lower availability of autocrine- or paracrine-acting vascular apelin could provide an additional or alternative explanation for this effect (Zhong *et al.*, 2007).

### Conclusion,

Acute apelin-13 administration in vivo caused NO-mediated decrease in mean arterial blood pressure, which was more significant in diabetic rats in comparison with normal and insulin treated rats.

In addition, apelin-13 injection antagonized the hypertensive action of Ang. II which was less significant in the diabetic group, and augmented the hypotensive effect of acetylcholine which was more significant in the diabetic rats in comparison with that of control and insulin treated groups.

Since different mechanisms are responsible for the diabetic vasculopathy 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. However, the impact of chronic administration requires further attention.

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