

# **SERUM ADROPIN LEVEL AND KIDNEY FUNCTIONS IN TYPE-II DIABETIC RAT MODEL WITH AND WITHOUT PIOGLITAZONE TREATMENT**

By

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## **ABSTRACT**

**Background:** Diabetic nephropathy (DN) is the most common cause of end stage renal disease (ESRD), and the leading cause of death in diabetic patients. Treatment with thiazolidinediones, particularly pioglitazone, may ameliorate kidney deterioration. Moreover, several peptide hormones participate in maintaining metabolic homeostasis including the recently discovered adropin. Data regarding adropin-circulating levels in type II diabetes mellitus (T2DM) are still conflicting.

**Objective:** This study was designed to determine pioglitazone effect on some metabolic and kidney function parameters in type II diabetic rat model, and to investigate the relationship between serum adropin and such parameters.

**Materials and methods:** Thirty healthy adult male albino rats were used for this study. The rats were randomly and equally divided into three groups. Group-1; control group, group-2; type II diabetic group, and group-3; pioglitazone- treated diabetic group. Rats were examined for body weight, body mass index (BMI), adropin, glucose & insulin, insulin resistance (HOMA-IR), lipids profile, inflammatory cytokines [interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- $\alpha$ ), plasminogen activator inhibitor-1 (PAI-1)], serum urea, creatinine, uric acid, angiotensin II, mean arterial blood pressure (MABP), urine flow rate, protein, creatinine, glomerular filtration rate (GFR), renal malondialdehyde (MDA) level, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities. Histopathological examinations for kidney tissues were also done.

**Results:** The present study revealed that diabetic rats showed significantly lower serum adropin levels than controls. Diabetes also significantly increased serum glucose with insulin resistance, serum total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-c), very low density lipoprotein-cholesterol (VLDL-c), serum IL-6, TNF- $\alpha$ , PAI-1, serum urea, creatinine, uric acid, angiotensin II, MABP, urine flow rate, protein and renal MDA [with significant negative correlation versus serum adropin] but significantly decreased serum insulin and high density lipoprotein-cholesterol (HDL-c), urine creatinine, GFR and renal SOD & GSH-Px activities [with significant positive correlation versus serum adropin] together with deterioration of kidney histoarchitecture. Moreover, pioglitazon treatment resulted in a significant recovery in the above mentioned parameters in the treated diabetic group.

**Conclusion:** Serum adropin concentration was negatively associated with the observed renal deterioration and may have a potential protective role, in addition to pioglitazone ameliorating effects, in diabetic nephropathy.

**Key words:** Diabetes, nephropathy, pioglitazone, adropin.

## INTRODUCTION

Diabetes is the most common cause of progressive kidney failure (nephropathy) that is reported to develop in 30–40% of patients with diabetes, and has become a leading cause of end stage renal failure worldwide (**Tanios and Ziyadeh, 2012**). Diabetic nephropathy is characterized by structural as well as functional abnormalities (**Forbes et al., 2008**).

The fundamental mechanisms responsible for nephropathy in diabetes involves tubulointerstitial fibrosis and glomerulosclerosis caused by hyperglycemia, hyperlipidemia, hypertension, oxidative stress, aldosterone–renin–angiotensin system activation, inflammation and release of profibrotic factors such as transforming growth factor  $\beta$ 1 & PAI-1 (**Qian et al., 2008 and Sarafidis, 2008**).

Currently available treatment options can only delay DN progression or turn to renal replacement therapies. Therefore, it is urgent to identify novel biomarkers for early diagnosis and to develop some effective treatment strategies for patients at high risk (**Hu and Chen, 2016**).

Thiazolidinediones [peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) agonists] in general, and pioglitazone in particular, reduce blood pressure and exert pleiotropic effects beyond glucose lowering in the human (**Abe et al., 2008**). In addition, adropin (ADR) is a new peptide metabolic hormone; 76 amino acid; expressed in the brain, liver, pancreas and kidney tissues, and has been shown to regulate energy homeostasis (**Kumar et al., 2008 and Aydin et al., 2013**). However, literature information showing the relationship

between adropin and T2DM is very limited although it can be an emerging diagnostic marker for diagnosis of T2DM (**Akcilar et al., 2016 and Hosseini et al., 2016**).

There are apparently contradictory reports concerning adropin-circulating levels in T2DM. **Hosseini et al. (2016)** reported higher adropin levels; meanwhile, others reported lower levels (**Butler et al., 2012 and Wu et al., 2014**) in T2DM patients. **Kumar et al. (2008)** have also found an association between adropin deficiency, increased adiposity and insulin resistance that could, therefore, be a factor regulating glucose and lipid homeostasis and can protect against the hepatosteatosis and hyperinsulinemia associated with obesity.

Therefore, we designed this study to examine the renal functions in type II diabetic rat model treated with pioglitazone, and identify the possible mechanisms of action in relation to serum adropin level.

## MATERIALS AND METHODS

Thirty healthy adult male wistar albino rats weighing 182–212 g were obtained from the animal house of Faculty of Veterinary Medicine- Zagazig University. Rats were kept in steel wire cages (70cm x 50cm x 30 cm- 5rats/cage) under hygienic conditions. They were fed the commercial rodent chow with free access to water, kept at room temperature and were maintained on a normal light/dark cycle. Rats were adapted to the new environment for one week before the experiment going on. The animal experiments were approved by the Institutional Research Board.

Rats were randomly divided into three equal groups:

Group-1 [normal control group]: in which rats were fed normal chow diet for 13 weeks.

Group-2 [Type 2 diabetic group rats]: in which rats were fed high fat diet (HFD) (60.3% of energy derived from fat, 18.4% from protein, and 21.3% from carbohydrates; 5.1 kcal/g) for 5 weeks, then the HFD was replaced with standard rodent diet and animals received single intraperitoneal injection of low dose of streptozotocin (STZ) (Sigma Aldrich Co.-USA) (35 mg/kg BW) dissolved in normal saline (Danda et al., 2005). Plasma glucose level was checked after 7 days of STZ injection. Rats with plasma glucose levels of  $\geq 250$  mg/ dl were included into the study for subsequent 13 weeks (Zhang et al., 2017).

Group-3 [Type 2 diabetic rats treated with pioglitazone]: Diabetic rats were given 12 mg/kg/day of pioglitazone HCL (ACTOS 30 mg tablets, Arab Pharmaceutical Manufacturing Co. Ltd, Sult - Jordan) in the chow for 13 weeks (Toblli et al., 2009).

#### **Anthropometric measures:**

**Measurement of body weight and length:** Each rat was put in closed plastic container and was weighed at the first and the last day of the experiment. The results were written in a record for each rat.

Body length was taken as the distance from the nose tip to the anus at the start and the end of experiment (Novelli et al., 2007).

#### **Calculation of Body Mass Index [BMI]:**

$BMI = \text{body weight (gm)} / \text{length}^2 (\text{cm}^2)$ .

(Novelli et al., 2007).

#### **Measurement of MABP pressure:**

MABP is measured in millimeters of mercury (mm Hg) by non invasive blood pressure monitor (NIBP 250, Serial No: 21202-108, BIOPAC system, Inc.; USA) (Abubakar et al., 2015).

**Urine collection:** Urine samples were collected for 24 hours by metabolic cages, measured for volume and centrifuged 10 minutes at approximately 3000 rpm to remove insoluble materials. The supernatant was kept at  $-20^{\circ}\text{C}$  for further analysis (Jones et al., 1973).

**Measurement of urine total proteins:** It was carried out as described by Nishi and Elin (1985) using urinary protein assay kit [Chondrex, Inc. 2607-151 place NE Redmond, WA 98052, USA].

**Measurement of urine Creatinine:** It was carried out as described by Jaffé (1986) using Creatinine (Colorimetric) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt].

**Blood sampling:** Retro-orbital venous plexus blood samples were obtained then serum was separated by allowing the blood samples to clot then centrifuged at 3000 rpm for 20 minutes, kept at  $-20^{\circ}\text{C}$  and used to measure the serum levels of adropin, glucose, insulin, lipids profile, IL-6, TNF- $\alpha$ , PAI-1, serum urea, creatinine, uric acid and angiotensin II.

#### **Measurement of serum adropin:**

Adropine was analyzed with rat adropine ELISA kit (Sun Red Biotechnology,

China- Catalogue No 201-11-3361) according to **Yang et al. (2016)**.

**Measurement of serum glucose and insulin:** Serum glucose was estimated as described by **Tietz (1995)** using specific glucose kit (Bioscience, Egypt) and analyzed by spectrophotometers device (URIT-810, China). Insulin was measured by enzyme amplified sensitivity immunoassay (EASIA) as described by **Temple et al. (1992)** using specific insulin kit (BioSource Belgium) and analyzed by spectrophotometers device.

**Calculation of Insulin resistance (HOMA-IR):** Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula [HOMA-IR = insulin ( $\mu$ U/mL) x glucose (mg/dl) /405] (**Matthews et al., 1985**).

**Measurement of serum lipids profile:** Total cholesterol (TC) and triglycerides (TG) were measured by enzymatic colorimetric method described by **Tietz (1995)** using specific cholesterol and triglycerides kits (Spinreact Spain), and analyzed by spectrophotometers device. High density lipoprotein-cholesterol (HDL-c) was measured by precipitating reagent method described by **Tietz (1995)** using HDLc precipitating reagent kit (Spinreact, Spain), and analyzed by spectrophotometers device. Low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) were estimated by using **Friedewald et al. (1972)** formula.

$$\text{LDLc} = \text{TC} - \text{HDLc} - \left( \frac{\text{TG}}{5} \right) \quad \text{VLDLc} = \frac{\text{TG}}{5}$$

**Measurement of serum creatinine:** It was carried out as described by **Jaffé (1986)** using Creatinine (Colorimetric) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt].

**Measurement of serum urea:** It was carried out as described by **Tietz (1990)** using urea/bun (urease) kit [Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia Egypt].

**Measurement of serum uric acid:** It was carried out as described by **Barham and Trinder (1972)** using uric acid (uricase/peroxidase) kit: [BioSystems S.A. Quality System certified according to EN ISO 13485 and EN ISO 9001 standards Costa Brava, 30. 08030 Barcelona, Spain].

Measurement of Glomerular filtration rate (GFR): By using creatinine clearance formula (Cockcroft and Gault, 1976).

$$\text{GFR}[\text{Creatinine clearance}](\text{ml}/\text{min}) = \frac{\text{Urine creatinine} \times \text{Urine volume}}{\text{Serum creatinine} \times 1440}$$

**Measurement of serum angiotensin II:** It was carried out as described by **Kumar et al. (2016)** using rat angiotensin II enzyme immunoassay (EIA) kit (Catalog Number EK-002-12; Phoenix Pharmaceutical Inc., Burlingame, CA).

**Measurement of serum TNF- $\alpha$  level:** It was carried out as described by **Fernando et al. (1998)**, using commercial ELISA kit, (Catalog Number RAB0480, provided by Sigma-Aldrich Co).

**Measurement of serum IL-6 and PAI-1 levels** were carried out as described by **Engvall and Perlmann (1971)**, using IL-6 ELISA Kit (Catalog Number RAB0306 provided by Sigma-Aldrich Co) and PAI-

1 ELISA Kit (serial No. 201-11-0637, Sunred biological technology, Shanghai, China).

**Tissue sampling and histopathological examination:** Immediately after collecting blood samples, rats were killed by decapitation after light ether anesthesia. Kidneys were immediately excised, the left one was processed for histopathological studies and the right one was homogenated for biochemical estimations of levels of MDA, SOD, and GSH-Px activities.

**Renal antioxidant system evaluation:** Tissue homogenates were prepared as described by **Zhang et al. (2017)**.

**Assay of MDA level** was according to the method described by **Ohkawa et al. (1979)**.

**Assay of superoxide dismutase (SOD) activity** was according to the method described by **Kakkar et al. (1984)**.

**Assay of glutathione peroxidase (GSH-Px) activity:** according to the method described by **Reddy et al. (1995)**.

**Histopathological examination** kidneys were rinsed with ice-cold normal saline (4°C) to exclude the blood cells, blotted and dried with filter paper, then tissue portions from kidneys were kept in 10% buffered formalin - saline at 4°C for at least one week (1ry fixation), then the specimens were dehydrated with a series of ascending grade of ethanol from 75 to 100%. Tissues were placed thereafter in xylol and embedded in paraffin wax. Cross sections of about 1-2 µm thickness of kidney was processed on slides and stained with haematoxylin and eosin (H &

E) stain to study general microscopic characters of the kidney for routine light microscope assessment at magnification power [400 ] (**Drury and Wallington, 1980**).

#### **Statistical Analysis:**

Results were presented as mean ± SD and analyzed using version 18 SPSS program (SPSS Inc. Chicago, IL, USA). One way Analysis of variance (ANOVA) was used followed by student- least significant differences (LSD) test to compare statistical differences between groups. Paired t. test was used to compare the initial and final body weights. Pearsons test was done to detect correlations between parameters. P value less than 0.05 was considered to be significant.

## **RESULTS**

The present study showed that HFD/STZ significantly increased serum glucose, HOMA-IR, serum TC, TG, LDL-c, VLDL-c, inflammatory cytokines (IL-6, TNF-α & PAI-1), serum creatinine, urea, uric acid, angiotensin II, urine flow rate and total urine protein, MABP, renal MDA levels. It significantly decreased final body weight, BMI, serum adropin, insulin, HDL-c levels, urine creatinine level, GFR and renal SOD, and GSH-Px activities in the diabetic group when compared to control (Tables 1-4 and photos 1-3).

However, It was found that pioglitazone treatment significantly decreased serum glucose, HOMA-IR, serum TC, TG, LDL-c, VLDL-c, inflammatory cytokines (IL-6, TNF-α & PAI-1), serum creatinine, uric acid, urea and angiotensin II, urine

flow rate, total urine protein, MABP, renal MDA levels. It significantly increased final body weight, BMI, serum adropin, HDL-c level, urine creatinine, GFR, renal SOD, and GSH-Px activities, with no significant change in serum insulin level in the treated diabetic group when compared to untreated diabetic one (Tables 1-4 and photos 1-3).

Moreover, there were significant negative correlations between serum adropin, serum glucose, HOMA-IR, serum TC, TG, LDL-c, VLDL-c, serum urea, uric acid, angiotensin II, urine flow rate, total urine protein, MABP and renal

MDA, but there were significant positive correlations between serum adropin, BMI, serum insulin, HDL-c, urine creatinine, GFR, renal SOD, GSH-Px activities in both diabetic and pioglitazone treated diabetic groups.

In addition, histopathological examination in the present study revealed severe damage to the kidney tissue including distorted renal glomeruli, vacuolated renal tubular cells, interstitial hemorrhage and severe cellular infiltration; in the diabetic group that was ameliorated by pioglitazone treatment in the treated diabetic group (photos 4-6).

**Table (1):** Body weights, serum adropin and final BMI of all studied groups

Parameters		Groups		
		Group -1	Group -2	Group -3
Initial body weight (g)	$\bar{X} \pm SD$	198.6 $\pm$ 8.44	196. 8 $\pm$ 10.03	197.6 $\pm$ 9.67
	P value of LSD		NS <sup>a</sup>	NS <sup>b</sup>
Final body weight (g)	$\bar{X} \pm SD$	279.8 $\pm$ 11.24	180.6 $\pm$ 7.05	208.2 $\pm$ 7.88
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a, b</sup>
	P value of t test between initial and final body weights	P < 0.001	P < 0.01	NS
Serum adropin (ng/ml)	$\bar{X} \pm SD$	37.9 $\pm$ 4.48	26 $\pm$ 4.37	31.1 $\pm$ 2.13
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a</sup> , P < 0.01 <sup>b</sup>
Final BMI (g/cm <sup>2</sup> )	$\bar{X} \pm SD$	0.56 $\pm$ 0.05	0.35 $\pm$ 0.02	0.40 $\pm$ 0.02
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a</sup> , P < 0.05 <sup>b</sup>
	r with serum adropin		r= 0.765, P < 0.05	r= 0. 740, P < 0.05

**a = versus group-1**

**b = versus group-2**

**Table (2):** Serum glucose , insulin, HOMA-IR, serum cholesterol, triglyceride, HDL-c, LDL-c and VLDL-c of all studied groups.

Parameters	Groups	Group I	Group II	Group III
	Serum glucose (mg/dl)	$\bar{X} \pm SD$	84.83±5.92	314.16±42.98
P value of LSD			P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
r with serum adropin			r= -0.979, P < 0.001	r= -0.977, P < 0.001
Serum insulin (?IU/ml)	$\bar{X} \pm SD$	21.121± 2.25	18.232±1.62	18.61±1.31
	P value of LSD		P < 0.01 <sup>a</sup>	P < 0.01 <sup>a</sup> , NS <sup>b</sup>
	r with serum adropin		r= 0.640, P < 0.05	r= 0.636, P < 0.05
HOMA-IR	$\bar{X} \pm SD$	4.41±0.49	14.04±1.59	8.73±0.79
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.663, P < 0.05	r= -0.63 <sup>∩</sup> , P < 0.05
Serum cholesterol (mg/dl)	$\bar{X} \pm SD$	75.586±5.49	170.44±13.08	112±11.96
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.940, P < 0.001	r= -0.677, P < 0.05
Serum triglyceride (mg/dl)	$\bar{X} \pm SD$	64.59±8.82	144.60±12.39	95.24±8.21
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.979, P < 0.001	r= -0.866, P < 0.01
Serum HDL-c (mg/dl)	$\bar{X} \pm SD$	39.99± 3.42	21.95±5.62	31.14±4.67
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a</sup> , P < 0.01 <sup>b</sup>
	r with serum adropin		r= 0.911, P < 0.001	r= 0.894, P < 0.001
Serum LDL-c (mg/dl)	$\bar{X} \pm SD$	22.67±8.17	119.56±15.99	61.8184±14.32
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.938, P < 0.001	r= -0.758, P < 0.05
Serum VLDL-c (mg/dl)	$\bar{X} \pm SD$	12.91±1.76	28.92±2.47	19.04±1.64
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.979, P < 0.001	r= -0.866, P < 0.01

**a = versus group-1**

**b = versus group-2**

**Table (3):** Serum TNF- $\alpha$ , IL-6 & PAI-1 and renal MDA levels, SOD, and GSH-Px activities of all studied groups.

Parameters		Groups	Group I	Group II	Group III
Serum TNF- $\alpha$ (pg/ml)	$\bar{X} \pm SD$		48.61 $\pm$ 4.58	66.20 $\pm$ 5.15	54.44 $\pm$ 3.89
	P value of LSD			P < 0.001 <sup>a</sup>	P < 0.01 <sup>a</sup> , P < 0.001 <sup>b</sup>
	r with serum adropin			r= -0.930, P < 0.001	r= -0.954, P < 0.001
Serum IL-6 (pg/ml)	$\bar{X} \pm SD$		9.73 $\pm$ 1.32	20.50 $\pm$ 2.53	13.87 $\pm$ 1.59
	P value of LSD			P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin			r= -0.901, P < 0.001	r= -0.836, P < 0.01
Serum PAI-1 (ng/ml)	$\bar{X} \pm SD$		4.68 $\pm$ 0.53	15.30 $\pm$ 2.59	9.03 $\pm$ 1.42
	P value of LSD			P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin			r= -0.776, P < 0.01	r= -0.821, P < 0.01
renal MDA(nmol/gm tissue)	$\bar{X} \pm SD$		37.27 $\pm$ 3.15	68.89 $\pm$ 5.48	52.07 $\pm$ 4.39
	P value of LSD			P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin			r= -0.947, P < 0.001	r= -0.935, P < 0.001
renal SOD activity (U/mg protein)	$\bar{X} \pm SD$		10.596 $\pm$ 1.14	6.21 $\pm$ 0.98	9.18 $\pm$ 0.84
	P value of LSD			P < 0.001 <sup>a</sup>	P < 0.01 <sup>a</sup> , P < 0.001 <sup>b</sup>
	r with serum adropin			r= 0.875, P < 0.01	r= 0.783, P < 0.01
renal GSH-Px levels (U/mg protein)	$\bar{X} \pm SD$		13.79 $\pm$ 1.29	9.25 $\pm$ 1.08	12.10 $\pm$ 1.33
	P value of LSD			P < 0.001 <sup>a</sup>	P < 0.01 <sup>a</sup> , P < 0.001 <sup>b</sup>
	r with serum adropin			r= 0.846, P < 0.01	r= 0.735, P < 0.05

**a = versus group-1****b = versus group-2**



**Table (4):** Serum creatinine, urea, uric acid, angiotensin II, urine flow rate, urine total proteins, urine creatinine concentrations, MABP and GFR of all studied groups.

Groups		Group I	Group II	Group III
Parameters				
Serum creatinine (mg/dl)	$\bar{X} \pm SD$	0.55±0.06	2.40±0.37	1.00±0.14
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.891, P < 0.001	r= -0.787, P < 0.01
Serum urea (mg/dl)	$\bar{X} \pm SD$	22.27±2.41	39.301±4.62	29.79±2.23
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.950, P < 0.001	r= -0.835, P < 0.01
Serum uric acid (mg/dl)	$\bar{X} \pm SD$	0.50±0.05	1.107±0.21	0.80±0.07
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.921, P < 0.001	r= -0.892, P < 0.01
urine flow rate (ml/24h)	$\bar{X} \pm SD$	9.85±0.84	15.07±1.25	11.69±1.20
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.01 <sup>a</sup> , P < 0.001 <sup>b</sup>
	r with serum adropin		r= -0.830, P < 0.001	r= -0.837, P < 0.01
urine total proteins (mg/24h)	$\bar{X} \pm SD$	53.42±5.51	293.43±21.16	108.91±14.36
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.950, P < 0.001	r= -0.959, P < 0.001
urine creatinine concentration (mg/ml)	$\bar{X} \pm SD$	124.39±9.16	92.45±11.20	105.61±8.90
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a</sup> , P < 0.01 <sup>b</sup>
	r with serum adropin		r= 0.964, P < 0.001	r= 0.986, P < 0.001
GFR (ml/min)	$\bar{X} \pm SD$	1.562±0.262	0.411±0.0819	0.865±0.120
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= 0.881, P < 0.01	r= 0.669, P < 0.05
MABP	$\bar{X} \pm SD$	92.41±4.67	134.9±6.04	115.89±6.19
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.001 <sup>a,b</sup>
	r with serum adropin		r= -0.795, P < 0.01	r= -0.807, P < 0.01
Serum angiotensin II (ng/?l)	$\bar{X} \pm SD$	0.257±0.031	0.407±0.052	0.315±0.036
	P value of LSD		P < 0.001 <sup>a</sup>	P < 0.01 <sup>a</sup> , P < 0.001 <sup>b</sup>
	r with serum adropin		r= -0.668, P < 0.05	r= -0.641, P < 0.05

**a = versus group-1**

**b = versus group-2**

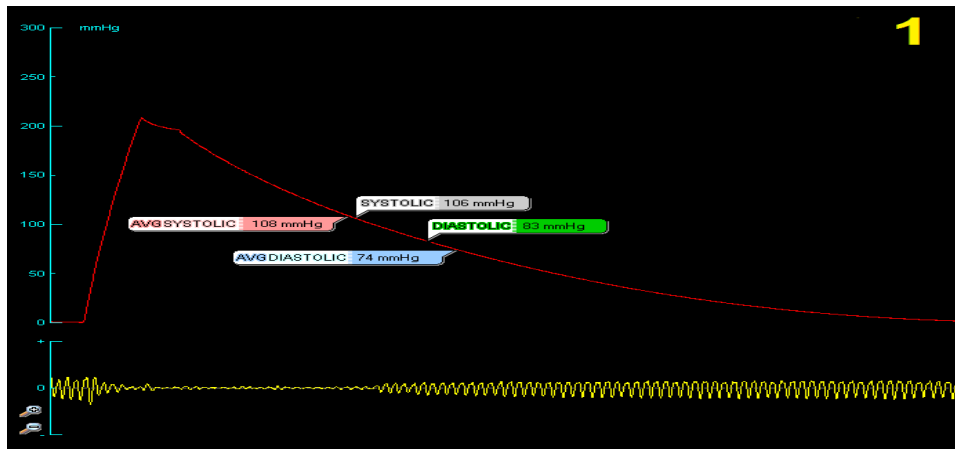


Photo (1): Recorded blood pressure from normal rats

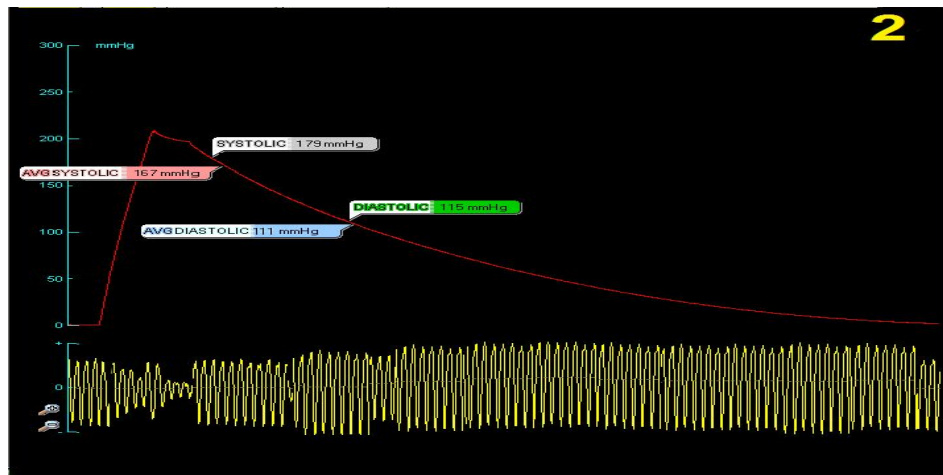
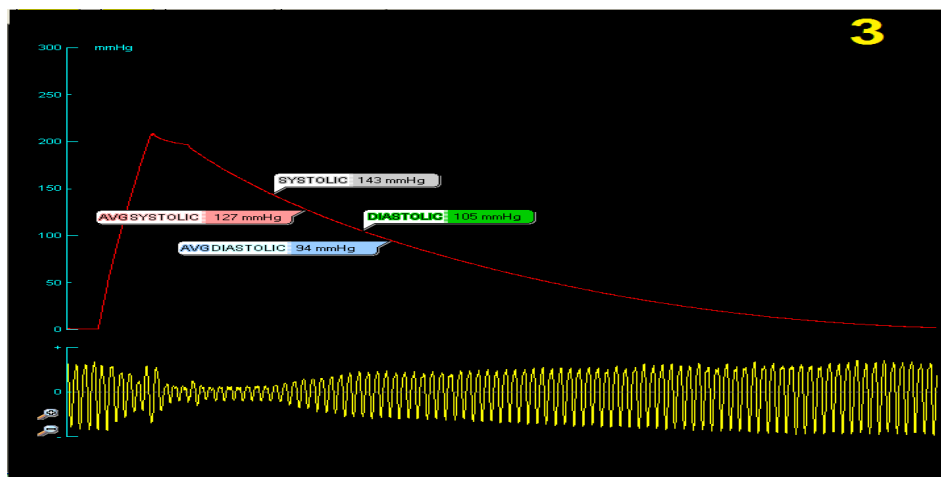
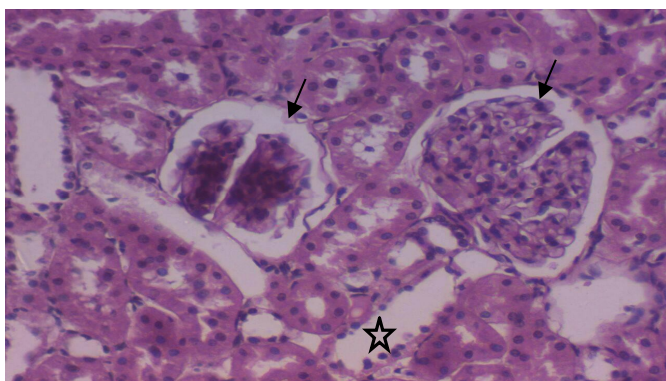


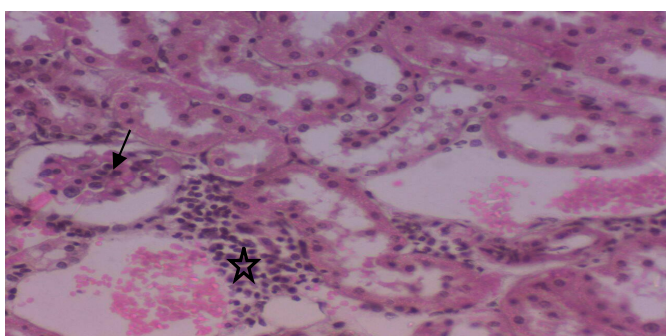
Photo (2): recorded blood pressure from rats in diabetic nephropathy group



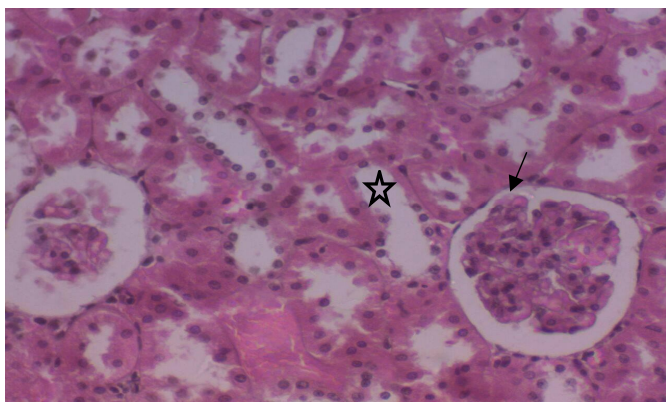
Photos (3): Recorded blood pressure from pioglitazone treated diabetic rats



**Photo (4):** Photomicrograph of kidney tissue of normal control group showing normal renal glomeruli (↑) surrounded by normal renal tubules (\*) (H&E X400).



**Photo (5):** Photomicrograph of kidney tissue of diabetic group showing distorted renal glomeruli (↑), vacuolated renal tubular cells, interstitial hemorrhage and severe cellular infiltration (\*). Apoptotic cells with pyknotic nuclei can also be observed (H&E X400).



**Photo (6):** Photomicrograph of kidney tissue of pioglitazone treated diabetic group showing improvement of renal architecture with minimal cellular infiltration and interstitial hemorrhage. Lining epithelium of renal tubules resumes to normal (\*). More or less normal glomeruli (↑) were also observed (H&E X400).

## DISCUSSION

The prevalence of type 2 diabetes mellitus (T2DM) has been increasing worldwide and more than two thirds of the patients may develop diabetic nephropathy (DN) (Xu et al., 2017). Current therapies for DN mainly including hypoglycemic agents and cotreatment with renoprotective drugs are not effective in stopping the progression of DN. Therefore, it is of vital importance and

urgency to find more effective therapeutic strategies in countering the diabetes-associated renal injury (Kong et al., 2017).

Treatment with adropin could reduce blood glucose levels and insulin resistance and improve insulin sensitivity in a rat model of T2DM (Akcilar et al., 2016). Also, data regarding serum adropin in T2DM remain conflicting (Wu et al., 2014 and Ugur et al., 2015). Thus, we

aimed in this study to examine the effects of pioglitazone on renal functions in type II diabetic rat model and identify the possible mechanisms of action in relation to serum adropin level.

High-dose STZ induced direct nephrotoxicity, making it unable to distinguish between the direct toxic effect of STZ and the lesions that resulted from STZ-induced hyperglycemia (**Tay et al., 2005**). In other studies, insulin resistance was induced in a rat model via a high-fat diet, followed by a single low dose of STZ to induce hyperglycemia (**Srinivasan et al., 2005 and Lee et al., 2013**). Elevated glucose levels after 13 weeks successfully induced renal lesions that were similarly present in human patients with DN (**Zhang et al., 2017**).

T2DM was confirmed in our study by hyperglycemia, hypoinsulinemia, and a significant increase in HOMA-IR index in the diabetic group. Whereas, pioglitazone treatment significantly decreased hyperglycemia and HOMA-IR index in the pioglitazone treated diabetic group when compared to the untreated diabetic one.

Hyperdyslipidemia is also a common symptom of DM (**Ma et al., 2014**). Significant increases in serum TC, TG, LDL-c and VLDL-c, in addition to a significant decrease in HDL-c were observed in the present work in the diabetic group. On the other hand, pioglitazone was found to significantly lower TC, TG, LDL-c and VLDL-c and significantly increased HDL-c concentrations in the treated diabetic group compared to untreated diabetic one. This was in accordance with the results of **Sendrayaperumal et al. (2014) and**

**Zhang et al. (2014) and** PPAR- $\gamma$  activation by pioglitazone controls a variety of genes involved in different pathways of lipid metabolism such as fatty acid uptake, fatty acid oxidation, lipolysis, and lipoprotein assembly and transport (**Grygiel-Gorniak, 2014**).

Decreased serum adropin level was also demonstrated in the diabetic rats. However, treatment with pioglitazone significantly increased that level. Moreover, the elevated serum glucose levels, HOMA-IR index, TC, TG, LDL-c and VLDL-c levels were correlated negatively to serum adropin level while the decreased HDL-c was positively correlated with it. This was in agreement with the studies of **Kumar et al. (2008) and Akcilar et al., (2016)** who also reported that therapy using synthetic adropin or transgenic over expression improves glucose homeostasis and dyslipidemia observed in T2DM. A significant increase in plasma adropin levels was also observed after Roux-en-Y gastric bypass, suggesting that reversal of the metabolic syndrome associated with obesity is also associated with a reversal of low plasma adropin levels (**Butler et al., 2012**).

However, in contrast to our results, **Aydin et al. 2013, Ugur et al. (2015), and Hosseini et al. (2016)** reported higher adropin levels in T2DM and argued that to the high glucose levels or a response to the anti-diabetic medications.

The discrepancy between our results and those of others may be due to species differences, duration of diabetes, and / or treatment used.

Moreover, our results revealed significant decreases in the GFR and urine

creatinine level together with significant increases in serum creatinine, urea, and uric acid levels, urine flow rate and protein in the diabetic group which indicates development of diabetic nephropathy. These results were in accordance with the findings of **Zhang et al. (2017)**, who reported that HFD/STZ-induced diabetes affect kidney function parameters after 13 weeks.

In addition, the elevated serum creatinine, urea, uric acid, urine flow rate and protein were correlated negatively to serum adropin level, while the decreased GFR and urine creatinine were positively correlated with circulating adropin level. This was in accordance with the results of **Wu et al. (2014) & Hu and Chen (2016)** who reported lower serum adropin concentrations in T2DM patients that were negatively correlated with blood creatinine, and positively correlated with glomerular filtration rate. However, **Hu and Chen (2016)** reported no significant correlation of serum adropin with dyslipidemia or blood glucose

Histopathological examination in the present study indicated severe damage to the kidney tissue including distorted renal glomeruli, vacuolated renal tubular cells, interstitial hemorrhage, and severe cellular infiltration. Noteworthy, treatment with pioglitazone successfully counteracted these morphological alterations and previous renal function parameters. This was in accordance with the findings of **Peng et al. (2014) and Chen et al. (2015)** who revealed that treatment with pioglitazone can reduce proteinuria and other manifestations of kidney damage.

Genetic variations that have subtle effects on the activity of gene encoding

adropin may confer increased susceptibility to type II diabetes (**Kumar et al., 2012**). Antidiabetic and hypolipidemic effects of adropin in streptozotocin-induced type 2 diabetic rats have also been previously demonstrated (**Akçinar et al., 2016**). Thus, its increase in the pioglitazone treated rats may play a role in the metabolic amelioration seen in that group.

Furthermore, diabetes in our study is associated with a significant reduction in the body weight. In agreement with our results, **Eissa et al. (2015)** reported that (HFD/ STZ) diabetic group showed an increase in body weight followed by acute weight loss after STZ injection. The body weight was progressively reduced due to hyperglycemia, hypoinsulinemia, increased muscle wasting and loss of tissue proteins (**Zafar & Naqvi, 2010 and Cheng et al., 2013**).

However, pioglitazone significantly increased the body weight in the treated group compared to diabetic group that may be through the improvement in glucose homeostasis, which was demonstrated in our results. Pioglitazone treatment may also increase feeding since PPAR- $\gamma$  increases feeding by reducing leptin levels, a hormone that regulates satiety center (**Brand et al., 2003**).

Chronic hyperglycemia results in the accumulation of advanced glycation end products, and increased production of reactive oxygen species (ROS) in renal tissue that plays a crucial role in the development of diabetic nephropathy through affecting lipids, DNA and proteins causing damage to the blood vessels and renal tissue (**Cheng et al., 2013 and Sohn et al., 2013**).

In our diabetic rat model, decreased levels of the SOD and GSH-Px activities accompanied by an increase in MDA levels were observed in the kidney tissue. However, treatment with pioglitazone ameliorates those values in the treated group. MDA is a late-stage lipid oxidation byproduct and an important indicator of free radical-induced lipid peroxidation (**Cheng et al., 2013**).

ROS further stimulates inflammatory cytokine production that serve as chemoattractants for macrophages and T-lymphocytes, and increase apoptosis of podocytes, endothelial and mesangial cells (**Bussolati et al., 2005**). They also cause increased production of vasoactive substances and reduced production of vasodilators such as prostacyclin and nitric oxide, which results in enhanced vasoconstriction by vasoconstrictors such as angiotensin II and PAI-1. These effects have significant vascular and renal consequences (**Keane, 2000**).

Our study also found significant increases in serum IL-6, TNF- $\alpha$  and PAI-1, with significant negative correlations versus serum adropin in the diabetic group. In accordance with our results, **Kume et al. (2016)** reported that circulating adropin level was negatively correlated with TNF- $\alpha$  level in women with polycystic ovarian syndrome. Adropin significantly decreased the mRNA expression levels of TNF- $\alpha$  and IL-6 in the pancreas of diabetic rats and may play a protective role in DN development through anti-inflammatory effects (**Akcilar et al., 2016 & Hu and Chen, 2016**).

Meanwhile, pioglitazone significantly decreased IL-6, TNF- $\alpha$  and PAI-1 serum levels in the treated diabetic group compared to untreated diabetic one. A low dose of pioglitazone, unable to normalize glycemia, also counteracts chronic inflammation and oxidative stress by blocking NF- $\kappa$ B pathway activation in the diabetic kidney (**Ko et al., 2008 and Rizos et al., 2008**). In vitro results also demonstrated that pioglitazone reduces glucose induced TGF- $\beta$ 1, fibronectin and collagen IV in human proximal tubular cells (**Panchapakesan et al., 2005**).

Angiotensin II (ANG II) also significantly increased in our study in the diabetic group compared to control, with significant negative correlation versus serum adropin level. This was in agreement with the finding of **Al-Qattan et al. (2016)** who reported a significant increase in plasma AngII in diabetic rats but at variance with that of **Vallon et al. (1995)** who reported decreased AngII concentration in diabetes.

In early DM, the concentrations of renal AngII and its receptor AT1 are reduced leading to hyperfiltration (**Nankivell, 2001**), but an augmented renal angiotensin system activity occurs at later stages of DM when molecular/cellular transformations lead to severe renal injuries; in particular glomerulotubular sclerosis, which impedes kidney clearance function leading to the end-stage renal failure that is characteristic of advanced DM (**Siragy et al., 2003**).

Hyperglycemia provoked high AngII concentration and stimulation of overexpressed AT1 receptors lead to

sodium retention, vascular resistance, glomerular capillary pressure, mechano-stretch-induced reactive oxygen species production, and tubulointerstitial cell hypertrophy and hyperplasia associated with extracellular mesangial matrix production (**Banday and Lokhandwala, 2008**).

MABP also significantly elevated in that study in the diabetic rats compared to control, but pioglitazone treatment significantly reduced it in the treated group. The reduction of blood pressure in our study may also have contributed to the renal protective effects of pioglitazone as prolonged systemic hypertension is an important contributor of the endothelial injuries to the kidneys (**Nazar, 2014**), although the converse process may also occur, e.g. an improvement of blood pressure due to pioglitazone beneficial effects on the kidney (**Toblli et al., 2009**).

The mechanism of hypertension in diabetic nephropathy includes excess sodium retention, sympathetic nervous system and renin-angiotensin-aldosterone system activation, endothelial cell dysfunction and increased oxidative stress (**Van Buren and Toto, 2011**). Decreased serum adropin levels are also negatively correlated with the increased blood pressure in our study.

**Gu et al. (2015)** also noted that adropin had a negative correlation with systolic and diastolic blood pressures in essential hypertension and may be an independent predictor for hypertension and may influence blood pressure by protecting endothelial function via increasing NO synthase expressions. Moreover, it may be

used as a marker indicating endothelial dysfunction in patients with T2DM (**Topuz et al., 2013**).

## CONCLUSION

Diabetes mellitus is associated with many deviations from normal homeostasis which include metabolic derangements (hyperglycemia and dyslipidemia), oxidative stress, inflammation, hypertension and fibrosis that lead to progression of DN. Moreover, decreased serum adropin level may have a role in the initiation of these effects.

Meanwhile, pioglitazone significantly improved diabetic nephropathy and all of the above mentioned deteriorations predisposing to its progression including the decreased circulating adropin level.

Further studies are required to investigate the use of adropin as a novel potential protective target for diabetic nephropathy.

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## مستوى الأدرابين في مصل الدم ووظائف الكلى في نموذج للجرذان المصابة بداء السكري من النوع الثاني مع وبدون العلاج ببوجليتازون

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**خلفية البحث:** يعد اعتلال الكلية السكري هو السبب الأكثر شيوعا لتدهور الكلى والوفاة في مرضى السكر، كما أن العلاج بتيازوليدينيون خاصة ببوجليتازون قد يؤدي إلى تحسن هذا التدهور. علاوة على ذلك، هناك العديد من الهرمونات التي تشارك في الحفاظ على التوازن الأيضي بما في ذلك الأدرابين المكتشف مؤخرا. ولكن لا تزال البيانات المتعلقة بمستويات الأدرابين في النوع الثاني من داء السكري متضاربة.

**الهدف من البحث :** تحديد تأثير ببوجليتازون على بعض معاملات التمثيل الغذائي ووظائف لكلى في نموذج للجرذان المصابة بداء السكري من النوع الثاني ، ودراسة العلاقة بين مستوى الأدرابين في مصل الدم وهذه المعاملات.

**مواد وطرق البحث:** تم إجراء هذه الدراسة على ثلاثين من ذكور الجرذان البيضاء البالغة التي يبلغ وزنها 182-212 جم وقد تم تقسيمها بشكل عشوائي الي ثلاث مجموعات متساوية: المجموعة الأولى التي استخدمت كمجموعة ضابطة ، و المجموعة الثانية هي المصابة بداء السكري من النوع الثاني ، والمجموعة الثالثة هي المصابة بداء السكري من النوع الثاني والمعالجة ببوجليتازون لمدة 13 أسبوع.

في نهاية فترة التجربة تم قياس وزن الجرذان وحساب مؤشر الكتلة ومتوسط ضغط الدم ومستوى أدرابين و السكر وهرمون الأنسولين ومقاومة الإنسولين في الدم ومستوى الكوليسترول الكلي ، الدهون الثلاثية والبروتين الدهني منخفض الكثافة جدا ومنخفض الكثافة وعالي الكثافة ومستوى انترلوكين 6 ومعامل نخر الورم الفاء، و مانع منشط البلازمينوجين-1 ومستوى الكرياتينين واليوريا وحامض اليوريك ومستوى أنجيوتنسين-2 في مصل الدم. كما تم قياس معدل تدفق البول وقياس مستوى البروتين والكرياتينين به ومعدل الترشيح الكبيبي باستعمال تركيز الكرياتينين في كل

من مصل الدم والبول. كما تم عزل الكلى وتحضير أنسجتها للدراسة الميكروسكوبية وقياس مستوى مالون داي الدهيد ومستوى نشاط الجلوتاثيون بيروكسيداز وسوبرأوكسيد ديسميوتاز بها.

**النتائج:** أدى داء السكري إلى نقص في تركيز الأدرابين في الدم. كما أدى إلى زيادة ذات دلالة إحصائية في كل من مستوى الجلوكوز ومقاومة الانسولين والكولسترول الكلي والدهون الثلاثية والبروتين الدهني منخفض الكثافة جدا ومنخفض الكثافة وانترلوكين ٦ ومعامل نخر الورم الفا و مانع منشط البلازمينوجين-١ والكرياتينين واليوريا وحامض اليوريك ومستوى أنجيوتنسين-٢ في الدم وحجم البول وتركيز البروتين به و مستوى مالون داي الدهيد في أنسجة الكلى [ مع وجود علاقة سلبية مقابل مستوى الأدرابين]، كما أدى إلى نقص ذو دلالة إحصائية في وزن الجسم ومؤشر كتلة الجسم ومعدل الترشيح الكبيبي والكرياتينين بالبول والبروتين الدهني عالي الكثافة وهرمون الإنسولين بمصل الدم ومستوى نشاط الجلوتاثيون بيروكسيداز وسوبرأوكسيد ديسموتاز في أنسجة الكلى في مجموعة السكري مقارنة بالمجموعة الضابطة [ مع وجود علاقة إيجابية مقابل مستوى الأدرابين] كما أدى داء السكري أيضا إلى تغيرات مرضية واضحة بالفحص المجهرى للأنسجة الكلوية في مجموعة السكري مقارنة بالمجموعة الضابطة، وقد تبين أن العلاج بببوجليتازون أدى إلى تحسن ذو دلالة إحصائية في المعاملات المذكورة أعلاه في مجموعة السكري المعالجة.

**الاستنتاج:** تركيز الأدرابين في مصل الدم يرتبط سلبا بالتدهور الكلوي، وقد يكون له دور وقائي بالإضافة إلى التحسن بببوجليتازون ضد إعتلال الكلية السكري.