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Protective Effect of Apelin-13 on Kidney and Platelet Functions in Adenine-Induced Chronic Renal Failure in Rats

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Abstract

Background: Apelin and angiotensin I receptor-related protein J receptor (APJ) were found to be expressed in the kidney. Also, in cases of Chronic Renal Failure (CRF), platelet function was found to be affected. Apelin-13 treatment effect on both kidney and platelet functions in cases of CRF was not fully understood.

Aim of Study: To investigate the possible protective effect of apelin-13 on kidney and platelet function in adenine-induced CRF in rats.

Material and Methods: Rats were divided into three groups of eight rats each as follow: Intact control group received a standard diet for 28 days, adenine treated group received a standard diet added to it adenine (0.75% w/w) for 28 days, and adenine + apelin treated group received a standard diet added to it adenine as in the adenine treated group with a concomitant treatment with apelin-13 for 28 days. Apelin-13 was dissolved in saline immediately before use and was given at a dose of 5μg/kg body weight/day subcutaneously. 24-hour urine samples were collected, and 24-hour water intake was estimated on the 28th day of the study, while each rat was kept in a separate metabolic cage. The collected urine samples were measured for volume, total protein, and creatinine levels. After 28 days from the start of the experimental period, rats were fasted overnight, and their blood pressure was measured using the Power Lab. Blood samples were collected and a part of it was placed in heparinized tubes to investigate platelet indices and another part of blood was kept in non-heparinized tubes which were left to clot for 30min at room temperature. Clotted blood was centrifuged at 3000rpm for 15min. The supernatant serum was pipetted off using fine tipped automatic pipettes and stored at –20ºC until assayed.

Results: Treatment with adenine (0.75%, w/w) for 28 days caused a significant decrease (p<0.001) in Body Mass Index (BMI) and a significant increase (p<0.001) of the relative kidney weight when compared with that of the control group. On concomitant treatment with apelin-13 and adenine significantly mitigated (p<0.001) the adenine-induced reduction in BMI and the increase in relative kidney weight. Water intake and urine output of adenine treated rats were significantly higher (p<0.001) than that in control rats, while, simultaneous treatment with adenine and apelin-13 significantly decreased (p<0.001) both in comparison to adenine treated group. Also, in comparison with control group, the serum levels of creatinine, uric acid and urea were enhanced significantly (p<0.001) in adenine treated group, indicating that adenine-induced CRF model has been successfully established in this experiment. However, these enhancements were significantly decreased (p<0.001) by concomitant treatment with apelin-13. On the other hand, there was a significant decrease (p<0.001) in the creatinine clearance in adenine treated group in comparison to the control one, however, simultaneous treatment with adenine and apelin-13 significantly deteriorated this effect. Also, in this study, comparing to those of normal group, the Superoxide Dismutase (SOD) and Glutathione Reductase (GR) levels in adenine treated group were significantly decreased (p<0.001) while, Malonaldehyde (MDA) was significantly increased (p<0.001). The alteration of SOD, GR and MDA serum levels was remarkably reversed by concomitant treatment with apelin-13. A significant increase (p<0.001) in platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and plateletcrit, but a significant decline (p<0.001) in bleeding time in adenine treated group in comparison to the control one. These changes were significantly reversed (p<0.001) by the concomitant treatment with adenine and apelin-13 in comparison to the adenine treated group.

Conclusion: Apelin-13 treatment improved both kidney and platelet functions, and may give a promising strategy for slowing the progression of CRF and its complications.

Key Words: *Chronic renal failure – Adenine – Apelin-13 – Platelet function – Plateletcrit – Rats.*

Introduction

APELIN is the endogenous ligand for the angiotensin I receptor-related protein J receptor (APJ) which is a seven transmembrane G protein-coupled receptor that has 31% amino acid sequence similar to angiotensin II type 1 receptor, but it doesn't bind angiotensin II [1]. Apelin and APJ are expressed in blood vessels, adipose tissue, lungs, heart, and kidneys [2-4]. Apelin is produced as a preproapelin

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formed of 77 amino acids, and then, cleaved by an angiotensin-converting enzyme to form several shorter bioactive peptides as apelin-13, -16, -17, -19 and -36 [5]. Apelin-13 is the most commonly used form because it has much stronger activity [6].The apelin/APJ system was found to be important in immunity, fluid and glucose homeostasis [7].Day, Cavaglieri [8] found that renal APJ expression and apelin- 13 levels were decreased in diabetic mice with nephropathy. Also, Han, Wang [6] declared that apelin levels decreased in rats with Chronic Kidney Diseases (CKD) and they attributed this to the vascular endothelial dysfunction caused by uremic toxins as the endothelium of vasculature is a vital source of plasma apelin [9]. Other hand, Wang, Diao [10] stated that apelin-13 improved kidney function in cases of CKD. Moreover, Chen, Li [11] found that apelin-13 had protective effects on diabetic nephropathy in mice. Chronic Renal Failure (CRF) is a major and growing public health problem that faces both developed and developing countries. It has been accompanied with bad health outcomes that include diminution in the length and/or quality of life [12]. Dialysis and kidney transplantation were the major methods of treatment of its end-stage, but difficult to afford. Therefore, there is a considerable interest in developing an effective treatment to prevent or delay CRF [13]. Evidence showed that inflammation and oxidative stress were implicated in the pathogenesis of CRF [14]. But, Pisarenko, Lankin [15] and Huang, Wu [3] found that apelin had anti-inflammatory and antioxidant actions. Thus, we speculated that apelin-13 could prevent or at least delay the development of CRF. In this study, adenine-induced CRF rat model was used as Yokozawa, Zheng [16] reported that it showed changes that resemble those occurred in CRF in humans. On the other hand, an increased platelet reactivity has been reported in patients with CRF [17,18]. On the contrary, Schoorl, Grooteman [19] found that there was a decrease in platelet function and an increase in the risk of hemorrhage in CRF. Moreover, Adams, Irish [20] declared that impaired renal function exposed patients to a higher risk of thrombosis. Shah, Oberweis [21] stated that platelet indices, including Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), plateletcrit and platelet count are indicators of platelet function and they are routinely reported in automated full blood counts. Thus, in this study, platelet indices were used as indicators of changes in platelet function in different study groups. Up to our knowledge, there is no study that investigate the possible protective action of apelin-13 on kidney and platelet function in adenine-induced CRF in rats, thus, this study was designed.

Material and Methods

This study was done from August 2017 to November 2017 in Zagazig Faculty of Medicine Physiology Department. Twenty-six adult healthy male albino rats, weighing 160-190gm were obtained from the Animal House of Faculty of Medicine Zagazig University. They were kept under hygienic conditions in steel wire cages (four per cage) in research laboratory of Faculty of Medicine Physiology Department. Rats were kept on diet consisting of mixed commercial rat laboratory chow and they had free access to water at room temperature and were maintained on a 12-hour light/dark cycle.

Experimental design:

After an acclimatization period of two weeks, rats were divided into three groups of eight rats each as follow: Intact control group received a standard diet for 28 days, adenine treated group received a standard diet added to it adenine (0.75% w/w) [16,22] for 28 days, and adenine + apelin treated group received a standard diet added to it adenine as in the adenine treated group with a concomitant treatment with apelin-13 for 28 days. Apelin-13 was obtained from Sigma-Aldrich, USA (Cat. No. A6469), dissolved in saline immediately before use and was given at a dose of 5 µg/kg body weight/day subcutaneously [23]. Adenine was obtained from Sigma (St. Louis, MO, USA). The standard diet consisted of 62.8% carbohydrate, 25.8% protein and 11.4% fat and it was obtained from Zagazig Faculty of Agriculture. In adenine treated group, 2 rats died, and 8 rats survived that period (death rate=[2/10] X 100=20%). During the treatment period, the rats were weekly weighed. Final Body Mass Index (BMI) was calculated on the 28th day of the study according to the equation:

$$\text{BMI} = \frac{\text{Body weight (gm)}}{\text{Lenght}^2\ (\text{cm}^2)\ \text{(from nose to anus length)}}$$ [24]

Also, 24-hour urine samples (urine output) were collected and 24-hour water intake was estimated on the 28th day of the study, while each rat was kept in a separate metabolic cage. The collected urine samples were measured for volume, total protein, and creatinine levels. Urinary levels of both total protein and creatinine were estimated using diagnostic kits (Chondrex.com, Cat. No. 9040 and Sigma-Aldrich, USA, Cat. No. MAK080, respectively). After 28 days from the start of the experimental period, overnight fasted rats' blood pressure was measured using the Power Lab (AD Instruments Pty Ltd, Australia) according to Parasuraman and Raveendran [25] and GERGES [26]. Blood samples were collected (about 3.5ml) from

the cannula after measuring blood pressure and a part of it (about 1.5ml) was placed in heparinized tubes [used to investigate platelet indices using Auto-Hematology Analyzer (MINDRAY-BC-2800, USA)] and another part of blood (about 2ml) was kept in non-heparinized tubes which were left to clot for 30min at room temperature **[27]**. The clotted blood was centrifuged at 3000rpm for 15min. The supernatant serum was pipetted off using fine tipped automatic pipettes and stored at –20_ until assayed. Platelet indices evaluated include platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and plateletcrit. Serum was investigated for creatinine, uric acid and urea by using diagnostic kits from Sigma-Aldrich, USA (Cat. No. MAK080, MAK077 and MAK006, respectively). Calculation of creatinine clearance according to the following equation:

$$\text{Creatinine clearance (ml/min)} = \frac{\text{Urine Cr (mg/dl) X urine volume (ml/day)}}{\text{serum Cr (mg/dl) X time (min)}} \quad \textbf{[28]}$$

Also, serum C-Reactive Protein (CRP) and Interleukin-6 (IL6) were measured using ELISA kits from Sigma-Aldrich, USA (Cat. No. RAB0097 and RAB03 12, respectively). Moreover, serum tumor necrosis factor- a (TNFα) and interleukin-10 (IL10) were estimated using ELISA kits from R & D Systems, USA (Cat. No. RTA00 and R1000, respectively). By decapitation under anesthesia, rats were killed. Both kidneys from each rat of each group were quickly collected, weighed, and washed with phosphate buffered saline, pH 7.4, that contained 0. 16mg/ml heparin for removal of any clots or red blood cells. One of the two kidneys from each rat of each group was wrapped in an aluminum foil to be stored at –80ºC to await biochemical analysis. For biochemical analysis, frozen renal tissues were thawed and homogenized in ice-cold Tris buffer (pH7.4) to give a 10% w/v homogenate which was centrifuged at 3000rpm at 4ºC for 15min **[29]** and the supernatant obtained was used to its contents of Superoxide Dismutase (SOD), Glutathione Reductase (GR) and malonaldehyde (malondialdehyde, MDA) using commercial kits which were purchased from Sigma-Aldrich, USA (Cat. No. 19160, GRSA and MAK085, respectively).

Relative kidney weight was calculated from:

$$\text{Relative kidney weight} = \frac{\text{Weight of kidney}}{\text{Rat weight}} \text{ X } 100\% \quad \textbf{[30]}$$

The other kidney from each rat of each group was fixed in dehydrated 4% paraformaldehyde in increasing ethanol concentrations, xylene cleared and paraffin embedded **[31]**. From the paraffin blocks, 5 µm sections were adjusted and stained with hematoxylin and eosin (H & E) using standard procedures. Bleeding time was assessed using a modified tail cutting method as described by Dejana, Villa **[32]**. The rats were put in a plastic cylinder that had several openings from one of them the animal's tail got out. Bleeding time was measured by transaction of tail; 2mm from the tip using a disposable surgical blade. With a filter paper, the cut was dabbed every 15sec until the paper was no longer stained red with blood. Bleeding time was then taken as the time when the blood stopped appearing from the cut tail.

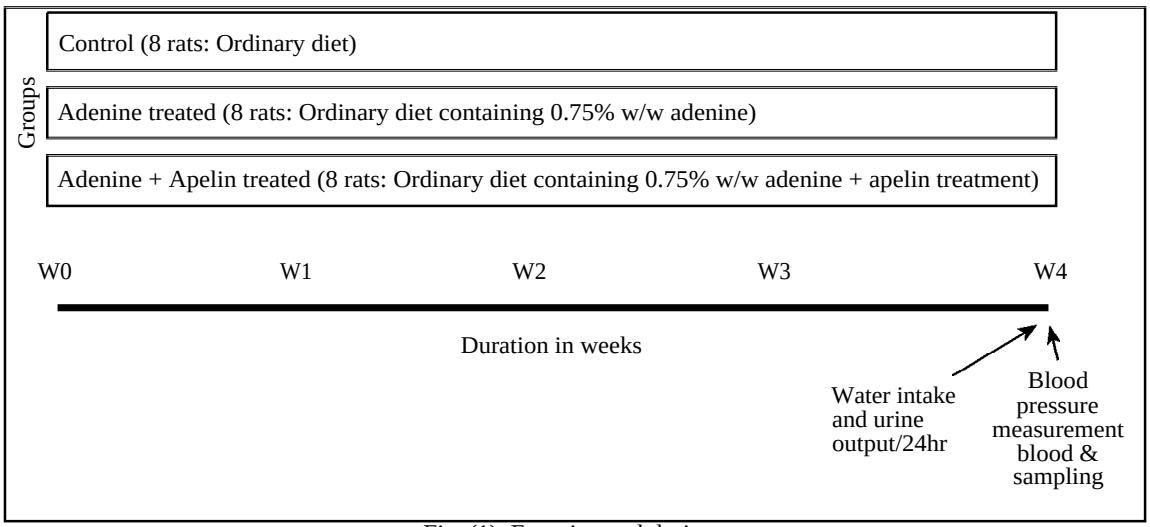


Fig. (1): Experimental design.

Statistical analysis:

The obtained data was expressed as mean values ± standard error of the mean (mean ± SEM). Means were compared by one-way analysis of variance (ANOVA) and Tukey HSD for post hoc Multiple Comparisons using (IBM SPSS Statistics Version 25 Software for Windows) for statistical significance. Correlation analysis using (Graph Pad Prism Version 7 Software for Windows) was done to investigate the relationship between MPV and the biochemical parameters that assessed the renal function in both adenine and adenine + apelin treated groups. p-value ≤0.05 indicated significance.

Results

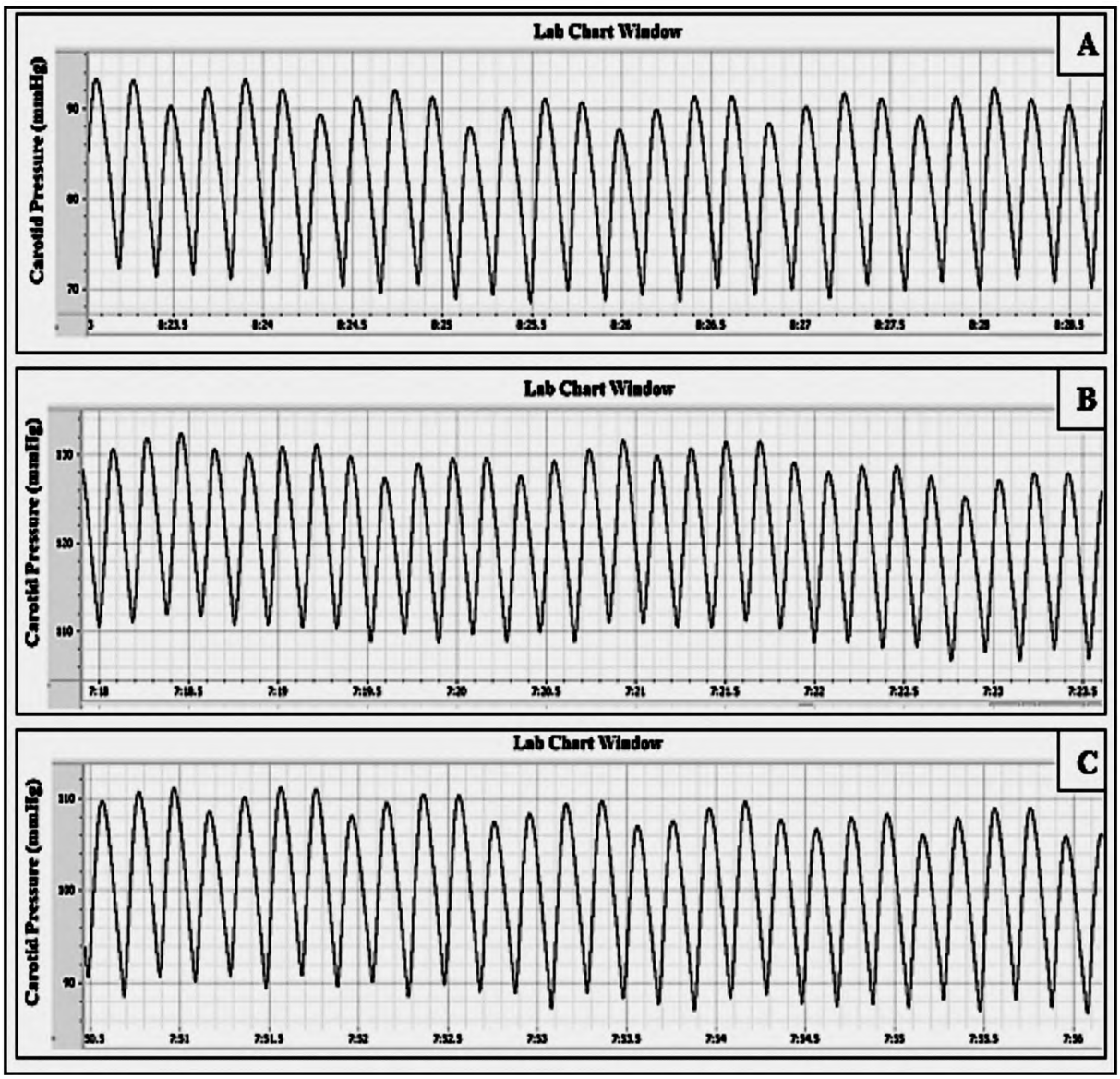
As noticed in (Table 1), treatment with adenine (0.75%, w/w) for 28 days caused a significant decrease (p<0.001) in BMI and a significant increase (p<0.001) of the relative kidney weight when compared with that of the control group. On concomitant treatment with apelin-13 and adenine significantly mitigated (p<0.001) the adenine-induced reduction in BMI and the increase in relative kidney weight. Water intake and urine output of adenine treated rats were significantly higher (p<0.001) than that in control rats, while, simultaneous treatment with adenine and apelin-13 significantly decreased (p<0.001) both in comparison to adenine treated group. Also, in comparison with control group, the serum levels of creatinine, uric acid and urea were enhanced significantly (p<0.001) in adenine treated group, indicating that adenine-induced CRF model has been successfully established in this experiment. However, these enhancements were significantly decreased (p<0.001) by concomitant treatment with apelin-13. On the other hand, there was a significant decrease (p<0.001) in the creatinine clearance in adenine treated group in comparison to the control one, however, simultaneous treatment with adenine and apelin-13 significantly deteriorated this effect. Proteinuria was analyzed as a marker of kidney injury, showing a significant (p<0.001) increase of excreted protein in adenine-treated rats, which was reduced significantly (p<0.001) by concomitant treatment with apelin-13. Also, in this study, comparing to those of normal group, the SOD and GR levels in adenine treated group were significantly decreased (p<0.001) while, MDA was significantly increased (p<0.001). The alteration of SOD, GR and MDA serum levels was remarkably reversed by concomitant treatment with apelin-13. These results suggested that the enhancement of antioxidant ability could be one of the mechanisms required for apelin-13 to protect renal function. Besides oxidative stress, inflammation was known as another vital etiological factor for the progression of CRF **[14]**. Therefore, the effects of apelin-13 on the inflammation of adenine-induced CRF rats were investigated.

Comparing with the control group, the serum levels of pro-inflammatory cytokines TNF-α, and IL-6 were significantly raised (p<0.001) in the adenine treated group, while the anti-inflammatory cytokine IL-10 was significantly reduced (p<0.001). However, the changes of these cytokines were significantly reversed by concomitant treatment with apelin-13. Also, CRP was significantly decreased (p<0.001) in rats treated with adenine + apelin when compared with rats treated with adenine alone. These results were consistent with the phenomenon that adenine-induced inflammatory cells infiltration was inhibited by concomitant treatment with apelin-13 as observed in the H & E staining Fig. (2). Thus, it was clear that the inflammation of CRF rats could be prevented by apelin-13.

To confirm the protective function of apelin-13 on the kidney, H & E staining were employed to analyze the effect of apelin-13 on the renal pathology of CRF rats. As shown in Fig. (2A), no sign of damage could be observed in the kidney section from normal group. In the adenine treated group Fig. (2B), kidney sections showed several signs of diffuse tubular injury and inflammatory cells infiltration. The renal pathological damages of CRF rats were remarkably decreased by concomitant treatment with adenine and apelin-13 Fig. (2C). On studying systolic and diastolic blood pressures in different groups (Table 1) and Trace (1), there was a significant increase (p<0.001) in their values in the adenine treated group in comparison to that in the control group. This effect was decreased significantly (p<0.001) by concomitant treatment with adenine and apelin-13. Table (2) showed a significant increase (p<0.001) in platelet count, MPV, PDW and plateletcrit, but a significant decrease (p<0.001) in bleeding time in adenine treated group in comparison to the control one. These changes were significantly reversed (p<0.001) by the concomitant treatment with adenine and apelin-13 in comparison to the adenine treated group. Also, in adenine treated group (Table 3), there was a significant positive correlation between MPV and each of serum creatinine (r=0.852, p<0.01), serum uric acid (r=0.888, p<0.01), serum urea (r=0.801, p<0.05), water intake/24hr (r=0.888, p<0.01), urine volume/24hr (r=0.981, p<0.001), protein in urine/24hr (r=0.814, p<0.05), serum TNFα (r=0.988, p<0.001), serum IL6

(r=0.817, p<0.05), serum CRP (r=0.907, p<0.01), MDA (r=0.817, p<0.05), systolic and diastolic blood pressures [(r=0.742, p<0.05) and **(**r=0.914, p<0.01)].

On the other hand, in the same studied group, there was a significant negative correlation between MPV and each of creatinine clearance (r=–0.756, p<0.05), serum IL10 (r=–0.816, p<0.05), SOD (r=–0.852, p<0.01) and GR (r=–0.847, p<0.01). On concomitant treatment with adenine and apelin-13, there was a significant positive correlation between MPV and each of serum creatinine (r= 0.858, p<0.01), serum uric acid (r=0.933, p<0.001), serum urea (r=0.953, p<0.001), water intake/24hr (r=0.771, p<0.05), urine volume/24hr (r=0.907, p<0.01) and protein in urine/24hr (r=0.850, p<0.01), serum TNFα (r=0.944, p<0.001), serum IL6 (r=0.93 1 , p<0.001), serum CRP (r=0.957, p<0.001), MDA (r=0.708, p<0.05), systolic and diastolic blood pressures [(r=0.756, p<0.05) and (r=0.945, p<0.001)]. On the other hand, in the same studied group, there was a significant negative correlation between MPV and each of creatinine clearance (r=–0.838, p<0.01), serum IL10 (r=–0.97, p<0.001), SOD (r=–0.949, p<0.001) and GR (r=–0.785, p<0.05).



Trace (1): Blood pressure of rats from the control (A), the adenine treated (B), and the adenine + apelin treated groups.

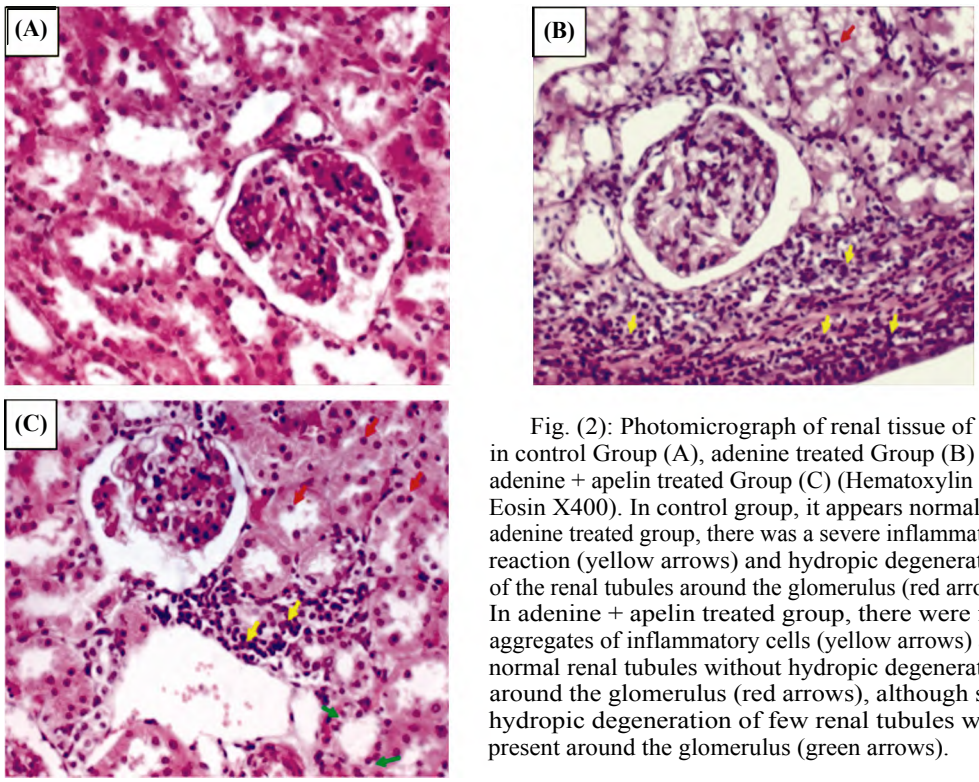


Fig. (2): Photomicrograph of renal tissue of rats in control Group (A), adenine treated Group (B) and adenine + apelin treated Group (C) (Hematoxylin and Eosin X400). In control group, it appears normal. In adenine treated group, there was a severe inflammatory reaction (yellow arrows) and hydropic degeneration of the renal tubules around the glomerulus (red arrow). In adenine + apelin treated group, there were few aggregates of inflammatory cells (yellow arrows) and normal renal tubules without hydropic degeneration around the glomerulus (red arrows), although still hydropic degeneration of few renal tubules were present around the glomerulus (green arrows).

Table (1): Biochemical, BMI and arterial blood pressure changes in different groups.

	Control	Adenine	Adenine + apelin
Final BMI (gm/cm^2)	0.52	0.48 **a**	0.52 **b**
Relative kidney weight (%)	0.53 ±0.02	1.6±0.04 **a**	0. 85±0.01 **a&b**
Water intake (ml/24hr)	20.55±0.18	51.2±0.24 **a**	34.33±0.24 **a&b**
Serum creatinine (mg/dl)	0.51 ±0.01	1.53±0.02 **a**	0.96±0.03 **a&b**
Serum uric acid (mg/dl)	1.25±0.01	3.28±0.02 **a**	2.37±0. 03 **a&b**
Serum urea (mg/dl)	27.36±0.64	72.27± 1.09 **a**	44.05±0.94 **a&b**
Serum C-reactive protein (µg/ml)	0.78±0.01	1.32±0.02 **a**	0. 85±0.02 **b&c**
Serum TNFα (pg/ml)	16.21 ±0.21	74.04±0.52 **a**	37.06±0.21 **a&b**
Serum IL6 (pg/ml)	35.25±0.29	160.39±0.63 **a**	99.88±0.24 **a&b**
Serum IL10 (pg/ml)	169.4±0.46	78.44±0.39 **a**	222. 84±2.27 **a&b**
Urine volume (ml/24hr)	7.96±0.14	37.26±0.63 **a**	22.21 ±0.5 **a&b**
Protein in urine (mg/24hr)	17.94±0.14	79.96±0.11 **a**	47.47±0.18 **a&b**
Urinary creatinine (mg/dl)	52.67±0.44	18.12±0.17 **a**	29.79±0.3 **a&b**
Creatinine clearance (ml/min)	0.58±0.01	0.31 ±0.01 **a**	0.48±0.02 **a&b**
SOD (U/gm)	1.34±0.01	0.86±0.01 **a**	1.18±0.01 **a&b**
GR (µg/gm)	7.27±0.01	4.73±0.02 **a**	6.18±0.01 **a&b**
MDA (nmol/mg)	0.64±0.01	1.81 ±0.01 **a**	1.24±0.01 **a&b**
Systolic blood pressure (mmHg)	98± 1.91	138.25±0.78 **a**	122.75± 1.1 **a&b**
Diastolic blood pressure (mmHg)	68.63± 1.56	98.75± 1.85 **a**	83.3 8± 1.1 **a&b**

Data was expressed as Mean ± SEM.
a : p<0.001 in comparison to control group.
b : p<0.001 in comparison to adenine treated group.
c : p<0.05 in comparison to control group.
BMI : Body Mass Index.
TNFα : Tumor necrosis factor alpha.
IL6 : Interleukin 6.
IL10 : Interleukin 10.
GR : Glutathione Reductase.
MDA : Malonaldehyde.

Table (2): Changes in platelet indices and bleeding time (sec) in different groups.

	Control	Adenine	Adenine + apelin
Platelet count (1000/mm^3)	214± 1.39	292.38±3.05 **a**	242. 13± 1.52 **a&b**
MPV (fl)	7.37±0.01	7.73±0.04 **a**	7.45±0.01 **b**
Plateletcrit (%)	0.18±0.001	0.216±0.002 **a**	0.189±0.001 **a&b**
PDW (fl)	8.59±0.01	9.76±0.02 **a**	8.65±0.02 **b&c**
Bleeding time (sec)	210.63± 1.93	177.5± 1.13 **a**	192± 1.46 **a&b**

Data was expressed as Mean ± SEM.
a: p<0.001 in comparison to control group.
b: p<0.001 in comparison to adenine treated group.
c : p<0.05 in comparison to control group.
MPV : Mean Platelet Volume.
PDW : Platelet Distribution Width.

Table (3): Pearson's correlation coefficient between MPV and biochemical parameters in both adenine and adenine + apelin treated groups.

Parameter	MPV			
	Adenine treated group		Adenine + apelin treated group	
	r	*p*	*r*	*p*
Serum creatinine	0.852	<0.01	0.858	<0.01
Serum uric acid	0.888	<0.01	0.933	<0.001
Serum urea	0.801	<0.05	0.953	<0.001
Creatinine clearance	–0.756	<0.05	–0.838	<0.01
Water intake/24hr	0.888	<0.01	0.771	<0.05
Urine volume/24hr	0.981	<0.001	0.907	<0.01
Protein in urine/24hr	0.814	<0.05	0.850	<0.01
Serum TNFα	0.988	<0.001	0.944	<0.001
Serum IL6	0.817	<0.05	0.931	<0.001
Serum IL10	–0.816	<0.05	–0.97	<0.001
Serum CRP	0.907	<0.01	0.957	<0.001
SOD	–0.852	<0.01	–0.949	<0.001
GR	–0.847	<0.01	–0.785	<0.05
MDA	0.817	<0.05	0.708	<0.05
Systolic blood pressure	0.742	<0.05	0.756	<0.05
Diastolic blood pressure	0.914	<0.01	0.945	<0.001

$p<0.05$ indicated statistical significance.

Discussion

The current study confirmed that adenine-fed rats had lower final BMI. This was supported by Ferrari, Ferreira [33] and Ali, Karaca [12] who referred this finding to polyuria, dehydration, uremia, and the poor palatability of adenine. Also, this study declared that adenine treatment significantly enhanced the relative kidney weight, as well as the water intake and urine output. This was supported by Ali, Karaca [12]. In agree with the results of this study, Ali, Alza'abi [22] found several signs of extensive damage and inflammation kidneys of adenine-treated animals. Also, Diwan, Mistry [27] stated that kidney damage in adenine-fed rats may be initiated by increased plasma uric acid levels.

Moreover, in agreement with our results, Ali, Alza'abi [22] found that adenine feeding caused significant increases in the concentrations of urea and creatinine in serum, but, a significant decrease of the creatinine clearance. These results were in agree with Han, Wang [6]. Also, results of this study showed presence of proteinuria that reflected proximal tubular dysfunction in adenine treated rats which was confirmed by Selvam, Kalaiselvi [34]. Inflammation and oxidative stress were known to be involved in the pathogenesis of CRF in rats [31]. Reactive oxygen species directly destroy mitochondrial function and protein synthesis [35]. In CKD, there was an increase in oxygen radical formation and a reduction in antioxidant defense [36]. In this study, in the adenine treated group, oxidative stress and inflammation markers were changed. There was a significant elevation in levels of MDA, TNFα, IL6 and CRP, but, there was a significant decline in SOD, GR and IL10 levels. These results were supported by Carrero and Stenvinkel [37], Standage and Wong [38], Ali & Al-Salam [39] and Ali & Karaca [12]. Also, our results were in agree with Koroglu & Akalin [40] who stated that values of the atherosclerotic risk factor CRP in patients with CKD were significantly increased than that in the control. Moreover, Dounousi & Papavasiliou [41] and Ali & Al-Husseni [31] found that oxidative stress was already found in early stages of renal disease and increased with declining kidney function. SOD and GR are important in the endogenous defense system to scavenge the free radicals and control the lipid peroxidation of free radicals. Also, the level of MDA indirectly reflect the oxidative damage severity induced by the free radicals [42]. Moreover, the current study showed a significant increase in both of systolic and diastolic blood pressures in adenine treated group in comparison to the control. This was in agreement with Lau, et al. [43] who declared that oxidative stress and inflammation observed in adenine induced CRF were the basis of the incidence of hypertension. On histopathological examination of kidneys of adenine-treated animals, tissue infiltration of white blood cells was observed as a sign of inflammation. This was supported by Ali & Al-Husseni [31] who stated that renal tissue of adenine treated rats showed proximal and distal tubules lesions, as well as in glomeruli. The increase in circulating pro-oxidant and pro-inflammatory mediators can induce platelets activation and enhanced thrombotic risk. However, in uremia, the prolonged stimulation can lead to platelet exhaustion [44]. Sharma, Thakur [45] stated that progressive interstitial fibrosis was involved in the pathogenesis of CKD. Also, Huang & Wu [3] confirmed that transforming growth factor-β1 (TGF-β1) induced Epithelial-Mesenchymal Transition (EMT) in proximal tubule epithelial cells which was essential in the pathogenesis of renal interstitial fibrosis that represented the final step of renal injury which ultimately leads to end-stage kidney failure, therefore, blocking TGF-β1 is an important way to prevent kidney injury. Wang, et al. [10] declared that apelin-13 inhibited TGF-β1-induced EMT and they suggested that apelin-13 may have potential therapeutic role for retardation of CKD progression. Also, Hus-Citharel, Bouby [46] stated that apelin-13 acted on pre- and postglomerular microvasculature to control kidney hemodynamics. Moreover,

Codognotto, Piccoli **[9]** found that in CKD patients, there was a significant decrease in apelin serum levels and they attributed that to uremic toxins induced vascular endothelial dysfunction. Also, Wang, et al. **[10]** speculated that the increased expression of APJ in the injured kidney may represent a compensatory response to overcome the downregulation of plasma apelin and progression of renal fibrosis. Thus, we postulated that co-administration of apelin- 13 with adenine may prevent or at least delay the deterioration in kidney function. The current study showed that 28 days' co-administration of apelin-13 and adenine to rats caused a significant enhancement of BMI, but, a significant decline in relative kidney weight in comparison to adenine treated group. This was supported by Chen, Wan **[23]** who demonstrated that apelin-13 treatment reduced kidney and glomerular hypertrophy. Also, in adenine + apelin treated group, our results showed a significant decline in levels of MDA, TNFct, IL6 and CRP, but, there was a significant increase in SOD, GR and IL10 levels in comparison to adenine treated rats. This was supported by Than, Zhang **[47]** and Pisarenko, Lankin **[15]** who stated that apelin-13 suppressed reactive oxygen species production and release. Also, they added that apelin-13 was able to relieve oxidative stress induced dysregulations of both expression of anti-and pro-oxidant enzymes, and, release of pro-and anti-inflammatory cytokines. In histopathological study of adenine + apelin treated rats, the inflammatory signs were significantly suppressed which was in agree with Chen, Wan **[23]** who stated that administration of 5μg/kg body weight of apelin-13 has protective effects on kidney morphology as it suppressed inflammation and apoptosis. On the other hand, MPV that measured platelet size was associated with platelet reactivity and was considered as a marker of platelet function **[48,49]**. This was confirmed by Chu, Becker **[50]** who declared that platelets became enzymatically and metabolically more active with increased MPV because they contained more prothrombotic materials as thromboxane A2. The current study confirmed a significant increase in MPV, but, there was a significant decline in bleeding time in adenine treated group in comparison to the control. These results were in agreement with Martinovic, Basic-Jukic **[17]**, Akpinar, et al. **[51]** and Ju, et al. **[52]** who stated that an increase in MPV increased platelet reactivity, shortened bleeding time and increased platelet aggregation. Also, Verdoia, et al. **[53]** found that patients with CRF showed a significant increase in MPV and they referred this increase to the swelling of platelets. Also, Chu, Becker **[50]** and Koroglu, et al. **[40]** confirmed the increase in MPV in CKD patients and they confirmed that MPV can be used as a biomarker to atherosclerosis risk in such cases. The mechanism related to enhanced MPV in CRF include presence of inflammatory reaction **[49,54]**. Moreover, Capan Konca, et al. **[55]**, Löf & Müller **[56]** and Li, et al. **[57]** confirmed the link between MPV, platelet activation, arteriosclerosis and inflammation as aggregated platelets released proinflammatory cytokines from alpha granules. In contrary to our results, Bessman, Gilmer **[58]** and Lokesh, Green **[59]** observed that MPV was low in patients with CRF. This discrepancy may be explained by the species difference. Moreover, our results showed a significant increase in platelet count, PDW and plateletcrit in adenine treated group in comparison to the control. These results were supported by Koroglu, et al. **[40]** who found that CKD patients had higher values of plateletcrit which was attributed to chronic inflammation that may increase the risk of atherosclerosis. Also, in agree with these results, Martin, Kristensen **[60]** and Ofem, et al. **[61]** declared that increased platelet count may contribute to the development and progression of atherosclerosis and the increase in arterial blood pressure. The increased platelet count in adenine treated group may be due to the increase in the proinflammatory cytokines which promote megakaryocyte proliferation as discussed by Klinger and Jelkmann **[62]**. Therefore, increased platelet count may reflect a proinflammatory condition with platelet activation **[63]**. On contrary, Salvati and Liani **[64]** and Schoorl, Grooteman **[19]** found that platelet count was decreased in end stage renal failure. This discrepancy was explained by difference in stage of CRF as in their studies, there was an end stage CRF with platelet exhaustion from repeated stimulation by false ligands as fibrinogen fragments. Plateletcrit indicated circulating platelets' number in a unit blood volume and it was directly related to the total number of platelets **[65]** while, PDW, the width of the curve of distribution of platelets related to the different sizes produced by these cells, is important for identification of platelet activity and was considered as a marker for increased blood coagulation **[66]**. The results of this study were supported by Lokesh, et al. **[59]** who stated that abnormal platelet indices was associated with an increase in the risk of thrombotic events among CRF patients. Also, the current study showed a positive correlation between MPV and serum creatinine levels, but there was a negative correlation with creatinine clearance. These results were supported by Ju & Kim **[52]** who found that MPV had a negative correlation with creatinine clearance and a positive correlation

with serum creatinine levels in patients with CRF. Moreover, our results showed a positive correlation between MPV and serum levels of CRP in the adenine treated group which was supported by Koroglu, Akalin **[40]**.

Conclusion:

Apelin-13 treatment improved the renal function of adenine-induced CRF in rats. The mechanisms of protection could be related to its anti-inflammatory, antioxidant and anti-fibrosis activities. On the other hand, in adenine treated group, there was a significant increase in platelet indices which reflected increased platelet activity that explained the thrombotic, atherosclerotic and arterial blood pressure changes in case of CRF. Also, there was an association between MPV and kidney functions which can also be used as an indicator for the platelet functions in CRF. Moreover, MPV can provide a favorable contribution to survival in CRF by causing prediction and treatment of development of inflammation and atherosclerosis. Furthermore, apelin- 1 3 co-treatment with adenine decreased platelet indices which reflected its protective effect on platelet function in CRF. Therefore, apelin- 13 treatment improved both kidney and platelet functions, and may give a promising strategy for slowing the progression of CRF and its complications. Further studies are requested to confirm these results.

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Conflict of interest:

None declared.

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التآثير الوقائى للآبيلين-١٣ على وظائف الكلى والصفائح الدموية فى الفشل الكلوى المزمن الناجم عن الآدينين فى الفئران

لقد وجد آن الآبيلين والذى هو عبارة عن هرمون بروتينى يتكون من ٧٧ حمض آمينى يفرز من آماكن مختلفة فى الجسم مثل الكلى ولقد تبين آيضا آن مستقبلاته متواجدة بالكلى. كما آوضحت آيضا العديد من الدراسات السابقة آن هذا الهرمون يؤثر على وظائف الكلى. وعلى الجانب الآخر آوضحت بعض الدراسات آنه فى حالات الفضل الكلوى المزمن تتغير وظائف الصفائح الدموية مما يؤدى إلى العديد من الآثار الجانبية مثل تكوين الجلطات آو حدوث تصلب للشرايين وإرتفاع فى ضغط الدم. وحيث آنه لم تكن هناك دراسة توضح تآثير الآبيلين-١٣ على وظائف الكلى والصفائح الدموية فى الفشل الكلوى المزمن الناجم عن الآدينين فى الفئران فلذلك تم تصميم هذه الدراسة.

وفى هذه الدراسة تم تقسيم فئران التجارب المستخدمة إلى ثلاث مجموعات:

المجموعة الآولى: الضابطة والتى تناولت الطعام المعتاد لمدة ٢٨ يوما.

المجموعة الثانية: معالجة بالآدينين حيث آنها تناولت الطعام المعتاد مضافا إلية الآدينين بنسبة ٠.٧٥٪ لمدة ٢٨ يوما.

المجموعة الثالثة: معالجة بالآدينين والآبيلين-١٣ حيث آنها تناولت الطعام المعتاد مضافا إليه الآدينين بنسبة ٠.٧٥ مع حقن تحت الجلد يوميا للآبيلين-١٣ ٥ميكروجرام لمدة ٢٨ يوما.

وبعد إنتهاء اليوم الثامن والعشرين تم قياس ضغط الدم ثم الحصول على الدم من الكانيولا المستخدمة آثناء قياس ضغط الدم وتم تقسيمه إلى جزئين:

- الجزء الآول تم وضعه بآنابيب خاصة لمنع حدوث التجلط (heparinized tubes) وتستخدم لدراسة الصفائح الدموية.
- الجزء الثانى تم وضعه فى آنابيب عادية وتركت لحدوث التجلط وتم فصل مصل الدم بإستخدام الطرد المركزى وتم تخزينه عند درجة حرارة- ٢٠ حتى وقت دراسته.

وآسفرت النتائج عن الآتى:

آولا: نقص ذات دلالة إحصائية فى مؤشر كتلة الجسم فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

ثانيا: زيادة ذات دلالة إحصائية فى مؤشر كتلة الجسم فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

ثالثا: زيادة ذات دلالة إحصائية فى كمية البول/٢٤ ساعة والماء المستهلك فى الشرب/٢٤ ساعة فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

رابعا: نقص ذات دلالة إحصائية فى كمية البول/٢٤ ساعة والماء المستهلك فى الشرب/٢٤ ساعة فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

خامسا: زيادة ذات دلالة إحصائية فى مستويات اليوريا والكرياتينين وحمض اليوريك وبروتين سى التفاعلى وعامل نخر الورم ألفا والأنترلوكين ٦ فى مصل الدم للفئران فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

سادسا: نقص ذات دلالة إحصائية فى مستويات اليوريا والكرياتينين وحمض اليوريك وبروتين سى التفاعلى وعامل نخر الورم ألفا والأنترلوكين ٦ فى مصل الدم للفئران فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

سابعا: نقص ذات دلالة إحصائية فى مستوى إنترلوكين ١٠ فى مصل الدم للفئران فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

ثامنا: زيادة ذات دلالة إحصائية فى مستوى إنترلوكين ١٠ فى مصل الدم للفئران فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

تاسعا: نقص ذات دلالة إحصائية فى تنقية الدم من الكرياتينين فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

عاشرا: زيادة ذات دلالة إحصائية فى تنقية الدم من الكرياتينين فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

حادى عشر: زيادة ذات دلالة إحصائية فى دلائل وظائف الصفائح الدموية ونقص فى وقت النزيف فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

ثانى عشر: نقص ذات دلالة إحصائية فى دلائل وظائف الصفائح الدموية وزيادة فى وقت النزيف فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

ثالث عشر: نقص ذات دلالة إحصائية فى مستويات نشاط (SOD) و (GR) بينما زيادة ذات دلالة إحصائية فى مستوى (MDA) فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

رابع عشر: زيادة ذات دلالة إحصائية فى مستويات نشاط (SOD) و (GR) بينما نقص ذات دلالة إحصائية فى مستوى (MDA) فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

خامس عشر: زيادة ذات دلالة إحصائية فى كل من ضغط الدم الإنقباضى والإنبساطى فى المجموعة المعالجة بالآدينين مقارنة بالمجموعة الضابطة.

سادس عشر: نقص ذات دلالة إحصائية فى كل من ضغط الدم الإنقباضى والإنبساطى فى المجموعة المعالجة بالآدينين والآبيلين مقارنة بالمجموعة المعالجة بالآدينين.

مما نستنتج أن الآبيلين-١٣ قد حسن من وظائف كل من الكلى والصفائح الدموية وذلك عند إستخدامه بالتزامن مع الآدينين مما يبرهن أن له دور وقائى على وظائف الكلى والصفائح الدموية فى الفشل الكلوى المزمن الناجم عن الآدينين فى الفئران وهناك الحاجة لمزيد من الدراسات لتأكيد تلك النتائج.