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 5 μg/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 in 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
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, Groteman [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:

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

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 Parassaranam 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 centrifuged at 3000rpm at 4°C 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:

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

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\( \mu \)m 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.](image-url)
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<0.001), 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=0.931, 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.
Table (1): Biochemical, BMI and arterial blood pressure changes in different groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Adenine</th>
<th>Adenine + apelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final BMI (gm/cm$^2$)</td>
<td>0.52±0.02</td>
<td>0.48±0.002</td>
<td>0.52±0.002</td>
</tr>
<tr>
<td>Relative kidney weight (%)</td>
<td>0.53±0.002</td>
<td>1.6±0.04$^a$</td>
<td>0.85±0.01$^a&amp;b$</td>
</tr>
<tr>
<td>Water intake (ml/24hr)</td>
<td>20.55±0.18</td>
<td>51.2±0.24$^a$</td>
<td>34.33±0.24$^a&amp;b$</td>
</tr>
<tr>
<td>Serum creatine (mg/dl)</td>
<td>0.51±0.01</td>
<td>1.53±0.02$^a$</td>
<td>0.96±0.03$^a&amp;b$</td>
</tr>
<tr>
<td>Serum uric acid (mg/dl)</td>
<td>1.25±0.01</td>
<td>3.28±0.02$^a$</td>
<td>2.37±0.03$^a&amp;b$</td>
</tr>
<tr>
<td>Serum urea (mg/dl)</td>
<td>27.36±0.64</td>
<td>72.27±1.09$^a$</td>
<td>44.05±0.94$^a&amp;b$</td>
</tr>
<tr>
<td>Serum C-reactive protein (µg/ml)</td>
<td>0.78±0.01</td>
<td>1.32±0.02$^a$</td>
<td>0.85±0.02$^b&amp;c$</td>
</tr>
<tr>
<td>Serum TNFα (pg/ml)</td>
<td>16.21±0.21</td>
<td>74.04±0.52$^a$</td>
<td>37.06±0.21$^a&amp;b$</td>
</tr>
<tr>
<td>Serum IL6 (pg/ml)</td>
<td>35.25±0.29</td>
<td>160.39±0.63$^a$</td>
<td>99.88±0.24$^a&amp;b$</td>
</tr>
<tr>
<td>Serum IL10 (pg/ml)</td>
<td>169.42±0.46</td>
<td>78.44±0.39$^a$</td>
<td>222.84±2.27$^a&amp;b$</td>
</tr>
<tr>
<td>Urine volume (ml/24hr)</td>
<td>7.96±0.14</td>
<td>37.26±0.63$^a$</td>
<td>47.47±0.18$^a&amp;b$</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>1.56±0.14</td>
<td>1.85±0.18$^a$</td>
<td>2.21±0.5$^a&amp;b$</td>
</tr>
<tr>
<td>Protein in urine (mg/24hr)</td>
<td>17.94±0.14</td>
<td>79.96±0.11$^a$</td>
<td>29.79±0.3$^a&amp;b$</td>
</tr>
<tr>
<td>Urinary creatinine (mg/dl)</td>
<td>52.67±0.44</td>
<td>18.12±0.17$^a$</td>
<td>22.75±1.1$^a&amp;b$</td>
</tr>
<tr>
<td>SOD (U/gm)</td>
<td>0.58±0.01</td>
<td>0.31±0.01$^a$</td>
<td>0.48±0.02$^a&amp;b$</td>
</tr>
<tr>
<td>GR (µg/gm)</td>
<td>1.34±0.01</td>
<td>0.86±0.01$^a$</td>
<td>1.18±0.01$^a&amp;b$</td>
</tr>
<tr>
<td>MDA (nmol/mg)</td>
<td>0.64±0.01</td>
<td>1.81±0.01$^a$</td>
<td>1.24±0.01$^a&amp;b$</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>98±1.91</td>
<td>138.25±0.78$^a$</td>
<td>122.75±1.1$^a&amp;b$</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.63±1.56</td>
<td>98.75±1.85$^a$</td>
<td>83.3±1.1$^a&amp;b$</td>
</tr>
</tbody>
</table>

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.
- $d$: p<0.05 in comparison to adenine treated group.

IL6 : Interleukin 6.
IL10 : Interleukin 10.
GR : Glutathione Reductase.
MDA : Malonaldehyde.
BMI : Body Mass Index.
TNFα : Tumor necrosis factor alpha.

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Adenine</th>
<th>Adenine + apelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (1000/mm$^3$)</td>
<td>214±1.39</td>
<td>292.38±3.05$^a$</td>
<td>242.13±1.52$^a&amp;b$</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>7.37±0.01</td>
<td>7.73±0.04$^a$</td>
<td>7.45±0.01$^b$</td>
</tr>
<tr>
<td>Plateletcrit (%)</td>
<td>0.18±0.001</td>
<td>0.216±0.002$^a$</td>
<td>0.189±0.001$^a&amp;b$</td>
</tr>
<tr>
<td>PDW (fl)</td>
<td>8.59±0.01</td>
<td>9.76±0.02$^a$</td>
<td>8.65±0.02$^a&amp;b$</td>
</tr>
<tr>
<td>Bleeding time (sec)</td>
<td>210.63±1.93</td>
<td>177.5±1.13$^a$</td>
<td>192±1.4$^a&amp;b$</td>
</tr>
</tbody>
</table>

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.
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, Doukoussis & Papavasiliou [41] and Ali & Al-Husseini [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-Husseini [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, Bousy [46] stated that apelin-13 acted on pre- and postglomerular microvasculature to control kidney hemodynamics. Moreover,

### 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, dehydratation, 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, Alz’aabi [22] found that adenine feeding caused significant increases in the concentrations of urea and creatinine in serum, but, a significant decrease in the creatinine clearance. These results were in agree with Han, Wang [6]. Also, results of this study showed presence of proteinuria that reflected the creatinine clearance. 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, Doukoussis & Papavasiliou [41] and Ali & Al-Husseini [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-Husseini [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, Bousy [46] stated that apelin-13 acted on pre- and postglomerular microvasculature to control kidney hemodynamics. Moreover,
patients with CRF showed a significant increase in MPV 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 present 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 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, TNF-ct, 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 agreement 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 Schoof, 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 CRF 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-13 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.

**Acknowledgements:**

To Professor Kamal El-Kashish, Pathology Department, Faculty of Medicine, Zagazig University for achieving the histopathological part of this study.

**Conflict of interest:**

None declared.

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التأثير الوقائي للأبيثين-13 على وظائف الكلي والصفائح الدموية في الفشل الكلوي المزمن الناجم عن الأدينين في الفئران

الإنجازات:

1. الهدف من الدراسة: 
- الدراسة مصممة لقياس وظائف الكلي والصفائح الدموية في الفشل الكلوي المزمن الناجم عن الأدينين في الفئران.

2. المواد والتقنيات المطبقة:
- تم تقسيم الفئران إلى ثلاث مجموعات:
  - المجموعة الأولى: الضباع بالماء، وتتألف من 28 قطعة.
  - المجموعة الثانية: ضروري ضعفًا بالكلي، حيث تتكون من 28 قطعة، وتتألف من 28 قطعة، وتتألف من 28 قطعة.
  - المجموعة الثالثة: ضروري ضعفًا بالكلي، حيث تتكون من 28 قطعة، وتتألف من 28 قطعة، وتتألف من 28 قطعة.

3. النتائج:
- بعد إتمام اليوم الثامن والعشرين، تم قياس ضغط الدم والحصول على أدمان الكلي بالمستحقة أثناء قياس ضغط الدم وتم تقسيمه إلى جزءين:
  - الجزء الأول: وضعه بانتياب خاص، منعت البلازما من الجلود الصلبة، وتم استخدام الطرد المركزي، وتم تخزينه عند درجة حرارة 20 درجة مئوية.

4. التوصيات:
- توصيات للدراسات القادمة:
  - توصيات ذات دلالة إحصائية في مؤسس كلي الجسم في المجموعة المعالجة بالأدينين مقارنة بالمجملية الضباع.
  - توصيات ذات دلالة إحصائية في مؤسس كلي الجسم في المجموعة المعالجة بالأدينين مقارنة بالمجملية الضباع.

الخلاصة:

- تأثير واسع النطاق للأبيثين على وظائف الكلي والصفائح الدموية في الفشل الكلوي المزمن الناجم عن الأدينين في الفئران.
Protective Effect of Apelin-13 on Kidney & Platelet Functions in Adenine-Induced CRF in Rats

خامسا: زيادة ذات دالة إحصائية في مستوى اليوبريك وحمض اليوبريك وبروتين سي التفاعل مع عامل نخر الوم أفا والأنترولونيين في مصل الدم للقنار في المجموعة المعالجة بالأندينين مقارنة بالمجموعة الضابطة.

سادسا: نقص ذات دالة إحصائية في مستوى اليوبريك وحمض اليوبريك وبروتين سي التفاعل مع عامل نخر الوم أفا والأنترولونيين في مصل الدم للقنار في المجموعة المعالجة بالأندينين والأبيزين مقارنة بالمجموعة المعالجة بالأندينين.

سابعا: نقص ذات دالة إحصائية في مستوى إنترولونيين في مصل الدم للقنار في المجموعة المعالجة بالأندينين والأبيزين مقارنة بالمجموعة الضابطة.

ثامنا: زيادة ذات دالة إحصائية في مستوى إنترولونيين في مصل الدم للقنار في المجموعة المعالجة بالأدينين والأبيزين مقارنة بالمجموعة المعالجة بالأدينين.

تاسعا: نقص ذات دالة إحصائية في نتائج عدد من الكرياتينين في المجموعة المعالجة بالأدينين مقارنة بالمجموعة الضابطة.

عاشرا: زيادة ذات دالة إحصائية في نتائج عدد من الكرياتينين في المجموعة المعالجة بالأدينين والأبيزين مقارنة بالمجموعة المعالجة بالأدينين.

حادي عشر: زيادة ذات دالة إحصائية في دليل وظائف الصفائح الدموية ونقص في وقت النزيف في المجموعة المعالجة بالأدينين مقارنة بالمجموعة الضابطة.

ثاني عشر: نقص ذات دالة إحصائية في دليل وظائف الصفائح الدموية وزيادة في وقت النزيف في المجموعة المعالجة بالأدينين والأبيزين مقارنة بالمجموعة المعالجة بالأدينين.

ثالث عشر: نقص ذات دالة إحصائية في مستويات نشاط (SOD) و (GR) في المجموعة (MDA) في المجموعة معالجة بالأدينين مقارنة بالمجموعة الضابطة.

رابع عشر: نقص ذات دالة إحصائية في مستويات نشاط (SOD) و (GR) في المجموعة معالجة بالأدينين والأبيزين مقارنة بالمجموعة المعالجة بالأدينين.

خامس عشر: زيادة ذات دالة إحصائية في كل من ضغط الدم الإنقباضي والانبساطي في المجموعة المعالجة بالأدينين مقارنة بالمجموعة الضابطة.

سادس عشر: نقص ذات دالة إحصائية في كل من ضغط الدم الإنقباضي والانبساطي في المجموعة المعالجة بالأدينين والأبيزين مقارنة بالمجموعة المعالجة بالأدينين.

مما نستنتج أن الأبيزين-13 قد حسن من وظائف كل من الكلي والصفائح الدموية وذلك عند إستخدامه بالتزامن مع الأدينين مما يبرهن أن له دور وقائي على وظائف الكلي والصفائح الدموية في الفشل الكلوي المزمن الناجم عن الأدينين في القنار، وهناك الحاجة لمزيد من الدراسات لتؤكد تلك النتائج.