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The Effect of Sildenafil on Adenine-Induced Chronic Kidney Disease in Mice

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Abstract

This study was done to find the potential renal protective effects of sildenafil and its underlying mechanisms in mice with adenine-induced CKD. For the experiment, 40 male mice were split into four groups. The control group (A) received the same food without medication until the research ends, while the other three collections (B, C, and D) were given adenine (0.25% w/w in feed daily for 8 weeks), groups (C and D) were given sildenafil (0.5 and 2.5 mg/kg) respectively orally every day for 30 days, and then blood samples were taken to assess the function of the kidneys (Urea, total protein, and creatinine), total antioxidant capacity (TAC), superoxide dismutase (SOD), and catalase (CAT) in addition to kidney histopathology, as well as body and kidney weight. Administration of adenine caused weight loss, increased TAC, SOD, and CAT, and increased plasma concentrations of urea, total protein, and creatinine. Damage-related renal histopathological indicators (fibrosis and inflammation) were considerably elevated by adenine. Most of the aforementioned metrics improved with sildenafil, indicating that it might be utilized as an adjuvant treatment for CKD in people through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms.

Keywords: sildenafil, adenine, nephroprotective, albino mice, antioxidant, histopathology.

تأثير السيلدينافيل على مرض الكلى المزمن الناجم عن الأدينين في الفئران

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قسم التقنيات الاحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

تهدف هذه الدراسة للتحقق في التأثيرات الوقائية الكلوية المحتملة للسيلدينافيل وآلياته الأساسية في الفئران المصابة بمرض الكلى المزمن الناجم عن الأدينين. تم فصل أربعين فأرًا من ذكور الفئران البيضاء إلى أربع مجموعات .المجموعة الضابطة (أ) تلقت نفس النظام الغذائي دون معالجة حتى نهاية البحث ، بينما عولجت المجموعات الثلاث الأخرى (ب ، ج ، د) بالأدينين (0.25٪ وزن / وزن في العلف يومياً لمدة 8 أسابيع) ، تم إعطاء المجموعتين C و D السيلدينافيل (0.5 و 2.5 مجم / كجم) فمويا على التوالي كل يوم لمدة 30 يومًا ، ثم تم أخذ عينات الدم لتقييم وظيفة الكلى (اليوريا ، والبروتين الكلي ، والكرياتينين) ، والقدرة الإجمالية لمضادات الأكسدة (TAC) ، ديسموتاز الفائق (SOD) ، والكتلاز (CAT) بالإضافة إلى الدراسة النسيجية

للكلى وكذلك وزن الجسم والكلى. أدى عقار الأدينين إلى خفض وزن الجسم ، و TAC ، و SOD ، و CAT، وزيادة تركيزات اليوريا والبروتين الكلي والكرياتينين. تم زيادة مؤشرات الأنسجة الكلوية المرتبطة بالضرر (الالتهاب والتليف) بشكل كبير بواسطة الأدينين. تم تحسين معظم المقاييس المذكورة أعلاه باستعمال السيلدينافيل ، مما يشير إلى أنه يمكن استعماله كعلاج مساعد لمرضى الكلى المزمن من خلال فعاليته في زيادة مضادات الأكسدة والآليات المضادة للالتهابات ومضادات موت الخلايا المبرمج .

1. Introduction

A long-term ailment, chronic kidney disease (CKD), is a significant and escalating public health issue in both industrialized and developing nations [1]. One in ten individuals globally, or 500 million people, are expected to have CKD, and this number rises to nearly half for those who are 75 or older [2]. Due to its high frequency of mortality and morbidity and the likelihood of developing end-stage renal disease (ESRD), CKD is extremely expensive for society [3]. Significant gonadal failure in both sexes is also a documented side effect of CKD. Patients with chronic kidney disease (CKD) do not currently have a single medication that may restore kidney function, and the only treatment measures available to reduce the disease's development are equalization of blood pressure, glucose levels, and insulin [4]. Therefore, there is a critical need for the discovery of innovative therapeutics to stop or reverse the decline in kidney function, particularly pharmaceuticals that have already been studied for other purposes. Oxidative stress, inflammation, and apoptosis are the pathophysiological causes of CKD and its side effects [5]. One of the most often used models is the adenine-induced model of CKD, which was initially introduced by Yokozawa et al. in 1986. Adenine is directly metabolized to 2,8-dihydroxyadenine, which can form crystals in the microvilli and the apical epithelial part of the proximal tubule. Within just two days after taking adenine, it resulted in a kidney injury. Growth retardation, which is more related to CKD, and an advanced course of CKD are caused by adenine, given in solution or diet, and its metabolite precipitating in the renal tubules [6]. Additionally, compared to the 5/6 nephrectomy type of CKD, this sample is simple to employ, has the lowest death rate in mice, does not require surgical expertise, and progresses in a manner comparable to that of human CKD [7]. Sildenafil citrate, labeled as Viagra, is a PDE-5 inhibitor that was first created to treat angina pectoris, but it has since been repurposed to cure erectile dysfunction and pulmonary hypertension. It exerts its effects by inhibiting the phosphodiesterase 5 enzyme, which causes the production of nitric oxide (NO) and cGMP [8]. Recently, it has undergone experimental testing for the treatment of various illnesses, including colon cancer and prostate cancer [9]. Sildenafil has been shown to dramatically decrease oxidative stress in the brains of stressed mice and the kidneys of rats with severe kidney damage and diabetic nephropathy that occurred because of cisplatin or ischemia-reperfusion injury [10]. Additionally, the medication has been proven to drastically decrease inflammatory demyelination and smoke-induced lung inflammation in mice [11]. It has also been noted that sildenafil can reduce renal tubular apoptosis in rats with a beta-amyloid burden as well as inhibit human first-stage trophoblast cells exposed to oxidative stress from undergoing apoptosis [12]. In this work, the renoprotective, anti-oxidative stress, and anti-inflammatory effects of sildenafil were assessed in mouse models of CKD.

2. Materials and Methods

2.1. Drugs, chemicals, and kits

Adenine, superoxide dismutase (SOD), total antioxidant capacity (TAC), and catalase (CAT) were purchased from Sigma Aldrich (Germany).

2.2. Laboratory animals

A total of forty male albino mice were utilized in this work at the age of 8–10 weeks with an average weight of 21±6 g. The mice were kept in a polypropylene crate under carefully controlled circumstances at a temperature of 25–28 °C with a 12-hour light/dark cycle. Mice were placed in acclimatization conditions for 14 days prior to the experiment.

2.3. Laboratory Design

40 male mice were at random separated into four collections (10 per collection).
Group A: The control group continued to extradite the same food without handling it until the end of the search.

Collections B, C, and D: mice from those collections were administered a powder food including adenine (0.25% w/w in diet) [13].
C and D groups: mice from Collections B, C, and D were administered sildenafil orally (0.5 and 2.5 mg/kg, respectively) [14]. in combination with the administration of normal nutrients.

2.4. Determination of Kidney and Body Weight

The kidney and body weight of the mice were determined using a compact electronic scale.
Samples: To test the parameters, blood samples were taken from the tail vein of mice. All blood was drawn into clean, dry tubes, either with or without EDTA.

2.5. Evaluation of renal function

Kidney functions were observed by tasting creatinine in the serum (Cat. no. 234-000), plasma urea nitrogen (Cat. no. UR 21-10), and total protein (Cat. no. 310-001) using ready-to-use assay kits (Schifgraben, Hannover, Germany) as instructed by the manufacturer. The above parameters are estimated using spectrophotometry [15].

2.6. Histopathological Study

Kidney tissue was fixed in 10% formalin for 24 hours before being dehydrated and embedded in paraffin. Approximately 4 µm-thick kidney strips were parted and spotted with hematoxylin and eosin (H&E). Light microscopic tests were shaped beforehand in 20 randomly selected regions in each part by observation [16].

2.7. Statistical Analysis

The impact of various factors on the study parameters was determined using the Statistical Analysis System (SAS) (2018) application. In this work, a significant comparison of means was made using the least significant difference (LSD) test (ANOVA) [17].

3. Results and Discussion

3.1. Renal function

Table 1 displays the impact of adenine and sildenafil therapy on plasma and serum indicators of renal function. Plasma concentrations of urea, total protein, and serum creatinine were all significantly ($P<0.001$) increased by adenine treatment compared with the control group. Sildenafil at the two graded doses used was significantly ($P<0.001$) decreased in these analyses compared with the adenine group. As the progenitor of signaling molecules such as adenosine and extracellular nucleotides, adenine is a purine nucleobase that is crucial for many biochemical and physiological processes in cells (ATP and ADP). Moreover, the kidney is one of several organs that express adenine receptors, which can function as an extracellular signaling molecule [18]. Later research showed that the adenine receptor is connected to the inhibitory G protein (Gi), which, when activated, suppresses the activity of adenylate cyclase

and results in a decrease in the generation of intracellular cAMP. The proximal tubule, collecting duct system, and thick ascending limb are the primary parts of the nephron that participate in the control of numerous kidney water and electrolyte homeostatic activities [19]. Adenine receptors interpose in the suppression of V2 receptor analogs and are expressed in glomerular capillaries, cerebral arterioles, and the collecting duct system. (1-deamino-8-D-arginine vasopressin; dDAVP) catalyzed cAMP generation in newly separated suspensions of internal medullary collecting system (IMCD) cells in mouse kidneys [20]. Antidiuretic hormone, or vasopressin, acts during its V2 receptor and cAMP/PKA-dependent route in the renal collecting system to regulate water equilibrium and fine-tune sodium homeostasis [21]. Moreover, patients with chronic renal illness had higher circulating levels of adenine, according to clinical trials [22]. Sildenafil works by boosting the repose of vascular smooth muscles and raising the level of cGMP in response to nitric oxide. PDE-5 is expressed and active in many tissues, such as the kidneys [23]. Sildenafil takes up the active place of phosphodiesterase type 5 (PDE5), providing high levels of cGMP, which starts smooth muscle relief and enhances blood circulation. Sildenafil prevents PDE5, resulting in cGMP aggregation, protein kinase G-1 (PKG-1) stimulation, and, in turn, PPAR-g stimulation to suppress TRPC6 expression. In podocytes, cGMP aggregation is known to inhibit kidney disease [24]. Numerous teams have researched in depth the elements of the pathways that cGMP buildup in podocytes activates, and there is a ton of evidence that TRPC6 inactivation is an effective strategy to treat kidney illness. For instance, the contractility, motility, and cytoskeletal organization of podocytes have all been associated with cGMP buildup [25]. Poor clinical prognosis in renal cell carcinoma is linked to PKG-1. It has been demonstrated that PPAR-g agonists shield podocytes from nephropathies, and only TRPC6 overexpression is enough to harm podocytes and lead to glomerulopathies [26]. In numerous kidney injury models, sildenafil therapy has significantly reduced proteinuria, oxidative stress, fibrosis, inflammation, hypertension, and overall renal dysfunction in a laboratory environment [27]. In comparison to the control groups, the treatment with sildenafil dramatically improved renal function indicators.

Table 1: Comparison between different groups in kidney functions

Group	Urea Mg/dl	total protein Mg/dl	Creatinine Mg/dl
Control	20.62 ±0.72 b	3.17 ±0.13 bc	135.74 ±1.91 b
Adenine	35.14 ±1.78 a	4.73 ±0.24 a	151.37 ±2.12 a
Sild 0.5	22.35 ±1.17 b	3.56 ±0.29 b	138.64 ±1.64 b
Sild 2.5	20.60 ±0.59 b	2.78 ±0.27 c	133.73 ±2.42 b
LSD value	3.497 **	0.737 **	6.127 **
P-value	0.0001	0.0003	0.0001

means that having the different letters in the same column differed significantly. ** (P≤0.01).

3.2. Effect of adenine and sildenafil on anti-oxidants

The impact of dealings with adenine and sildenafil on certain antioxidant parameters is seen in Table 2. Adenine significantly ($p < 0.01$) decreases SOD, TAC activities, and CAT, showing that the kidneys may experience substantial oxidative stress due to adenine. Sildenafil treatment significantly increased ($p < 0.01$) the antioxidant-related markers SOD,

TAC, and CAT at the two doses. Inflammation, apoptosis, nitrosative, and oxidative stress are famously common in CKD, which is probably the inferior cause of subsequent hypertension and different side effects [28]. The indicators in urine, plasma, and kidney homogenates were treated with adenine, which had the anticipated and previously documented effects of oxidative stress, inflammatory effects, and apoptosis [29]. Long-term ingestion of adenine can cause it to oxidize into dihydroxyadenine, and the dihydroxyadenine crystals may increase the generation of harmful superoxide anion radicals and peroxides, resulting in ROS accumulations [30]. The imbalance between the production and elimination of ROS leads to oxidative stress, which can harm the kidney. Increased free radicals and oxidants can cause this [31]. By impairing the stimulation of nuclear factor erythroid 2-related factor 2 (Nrf2), an activator that controls the expression of genes encoding antioxidant and detoxifying proteins and enzymes like SOD, TAC, CAT, and NADPH, therapies with adenine resulted in a reduction in the anti-oxidants in plasma [32]. As a result of the sildenafil administration, the antioxidant index plasma concentration was found to be considerably higher, according to our findings. These results back up the claims that sildenafil has potent anti-inflammatory and antioxidant characteristics [33]. It is possible to use sildenafil to lessen healing time and stop oxidative damage. Numerous studies have documented sildenafil's antioxidant effects in disease simulations via the Nrf-1/HO-1 cytoprotective system [34]. In mice with CKD, sildenafil was found to reduce oxidative stress and DNA damage. Moreover, CKD can be avoided by maintaining oxidant/antioxidant balance and lowering TNF-a levels [35]. It was discovered that sildenafil inhibits pro-inflammatory cytokines like TNF and activates antioxidant defense genes such as heme oxygenase-1 (HO-1) and NAD (P) H: quinone oxidoreductase 1 (NQO1). It also attenuates pro-inflammatory cytokines like TNF and protects against renal ischemia-reperfusion injury [36].

Table 2: Comparison of different groups of antioxidants

Group	SOD (U/mg)	TAC (nmol/µl)	CAT (nmol/µl))
Control	18.89 ±1.02 ab	0.882 ±0.20 a	119.68 ±7.15 a
Adenine	12.31 ±0.67 c	0.028 ±0.01 b	74.09 ±1.81 c
Sild 0.5	17.38 ±0.40 b	0.636 ±0.23 a	95.29 ±3.83 b
Sild 2.5	20.22 ±0.56 a	1.052 ±0.01 a	132.07 ±6.71 a
LSD value	2.119 **	o.461 **	16.018 **
P-value	0.0001	0.0013	0.0001

means having the different letters in the same column differed significantly. ** (P≤0.01).

3.3. Effect of adenine and sildenafil on Kidney and Body Weight

Table 3 shows that low body weight significantly ($p < 0.01$) increased after adenine feeding compared with the control group. Adenine is linked to decreased food intake through polyuria; after adenine induction therapy, weight loss may be caused by renal tubule injury and a subsequent decrease of renal tubular cells that can absorb water, which causes dehydration and weight loss [37]. The lowering in protein production of the top water canal

AQP2 in the collecting duct across the kidney correlates with an increase in urine production and a reduction in urine osmolality. This impact is correlated with a decrease in Na-K2-Cl cotransporter (NKCC2 or BSC1) protein expression in the medullary thick ascending limb [38]. Both AQP2 and NKCC2 had considerably decreased mRNA expression, suggesting that the impacts of adenine may affect the stability of mRNA or modifications in the transcription of the genes encoding these proteins. Down-regulation of AQP2 and NKCC2 in response to adenine is related to subsequent fluid loss [39]. Adenine causes a considerable reduction in food intake when combined with continuous food restriction. Research has revealed that the adenine receptor is also found in the intercalated cells (IC) of the collecting duct system and the hypothalamus. Whether the changes in eating behavior are caused by the stimulation of this receptor by adenine in the IC and hypothalamus [40], the progression of early prerenal failure to CKD in a lengthy period of adenine eating is likely influenced by the interaction of renal fluid loss, reduced food intake, and ultimately huge volume depletion [41]. In this study, the concentration (0.5 mg/kg) of sildenafil did not affect the body weight, and the concentration (2.5 mg/kg) of sildenafil significantly ($p < 0.01$) elevated body weight after adenine feeding, as shown in Table 3. Phosphodiesterase 5 inhibitors have recently been found to have insulin-sensitizing, thermogenic, and weight-reducing effects, suggesting that increasing intracellular rates of cyclic guanosine monophosphate (cGMP) may be a viable treatment for metabolic diseases [42]. The use of a PDE5 inhibitor was able to reduce the expression of inflammatory markers, which is a key factor in the development of obesity. Consequently, long-term sildenafil citrate therapy may prevent weight gain through many methods other than lipolysis of fat cells. Additionally, phosphodiesterase-5 inhibition boosted energy expenditure, indicating improved energy balance and weight loss [43]. Regarding kidney weight, adenine caused a non-significant ($p < 0.01$) rise in kidney weight compared to the control collection. The in vivo mouse studies revealed that adenine might result in biochemical, histological, and molecular abnormalities in the CKD mice, involving fibrosis, inflammation, oxidative stress, and other conditions [44]. This increase may be due to these changes in the kidney tissue. Treatment with sildenafil at two doses caused a non-significant ($p < 0.01$) decrease in kidney weight compared with the adenine group. According to reports, sildenafil inhibits the expression of inflammatory cytokines in the tissues of the kidney, liver, and brain. Additionally, it lessens endothelial-leucocytic contact, inflammatory cell recruitment, and infiltration [45]. The decreased weight of the kidneys following sildenafil treatment may be due to the drug's ability to reduce the number of fibrosis and inflammatory cells in CKD.

Table 3: Comparison between different groups in body and kidney weight

Group	Body weight (gm)	Kidney weight (gm)
Control	26.68 ±0.71 a	0.440 ±0.05 b
Adenine	14.82 ±0.67 c	0.449 ±0.08 b
Sild 0.5	14.52 ±0.89 c	0.448 ±0.06 b
Sild 2.5	16.70 ±1.07 c	0.402 ±0.02 b
LSD value	2.556 **	0.176 **
P-value	0.0001	0.0001

means having the different letters in the same column differed significantly. ** (P≤0.01).

3.4 Histopathology

The modifications of histopathological kidney slices were observed in H&E-stained slices. In the collection that was provided with adenine, histopathological changes involved necrosis, apoptosis, and inflammation. Figure 2 Adenine management has the potential to make oxidative stress worse, and prior research has shown that oxidative stress can cause apoptosis and DNA damage in the kidney. Oxidative stress, inflammation, and apoptosis are known to be common in CKD [46]. Caspases are the primary factors of apoptosis, which has been demonstrated to be induced by DNA damage. The primary descending caspase of apoptosis, Caspase-3, might cause cell death via mitochondria-dependent (Bax/Bcl-2) pathways. It is implicated in various types of apoptosis that cause apoptosis [47]. On the one hand, the disparity between the pro-apoptotic factor (Bax) and anti-apoptotic agent (Bcl-2) causes apoptosis by caspase stimulation. On the other hand, the triggered Caspase-3 might control the proportion of Bax/Bcl-2 and transfer the Bax/Bcl-2 in a pro-apoptotic path. Additionally, studies have shown that cell apoptosis is a significant contributor to renal fibrosis [48]. The fibrosis seen in this research may have been caused by inflammation. Inflammatory cells generate a number of growth factors and cytokines, including TNF-a and interleukin (IL)-1b, which are often implicated in fibrosis via the production of adenine, leading to an increase in mice with CKD, according to our findings [49], compared to control mice. Figure 1 Our results indicated that sildenafil treatment significantly increased the plasma concentration of the antioxidant. These impacts support reports that sildenafil is a potent anti-inflammatory and antioxidant factor [50]. In numerous kidney injury models, sildenafil therapy has proven generally useful in lowering inflammation, oxidative stress, proteinuria, fibrosis, hypertension, and overall renal damage. According to reports, sildenafil inhibits the representation of inflammatory cytokines in the tissues of the kidney, liver, and brain. Additionally, it lessens endothelial-leucocytic contact, infiltration, and inflammatory cell recruitment [51]. One of the main pathophysiological alterations in renal damage is renal apoptosis. In reaction to renal ischaemia, caspase-3 is the last stage of the apoptotic cycle and starts the morphological cascades of apoptosis. According to reports, sildenafil reduces the number of apoptotic cells [52]. A statistically significant decrease in caspase-3 expression was linked to sildenafil's stronger anti-apoptotic impact, which leads to enhanced expression of the Bcl-2 protein, which is the cause of sildenafil's anti-apoptotic effects. The intrinsic process of apoptosis is inhibited when the Bcl-2 protein is activated because it down-regulates the Bax protein [53]. Additionally, sildenafil enhances renal hemodynamics and increases NO expression in renal tissue. This is accomplished by up-regulating eNOS mRNA expression while down-regulating inducible NOS mRNA production in renal tissue [54], as shown in Figure 3 with 2.5 mg/kg, while these changes were light in the tissue of the kidneys of mice treated with a dose of 0.5 mg/kg. Figure4.

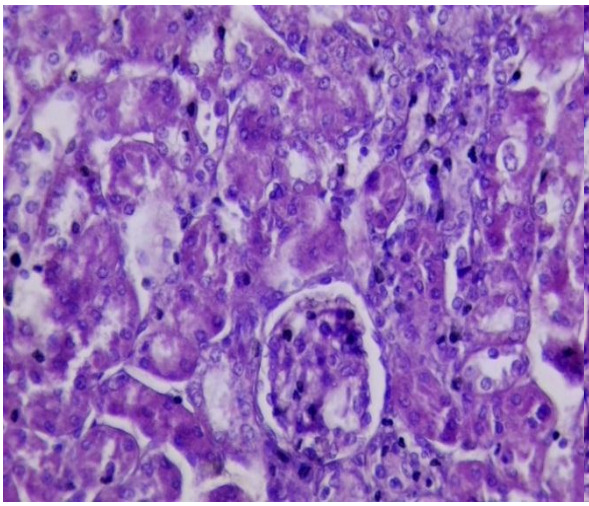

Figure 1: Histological sections in mice's kidneys from the control collection (H&E stain) (X400)

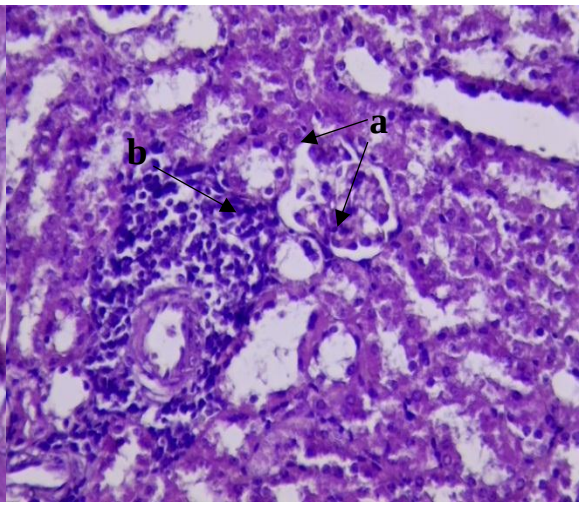

Figure 2: Histological sections in mice's kidneys of the adenine collection showed a: necrosis, b: inflammation (H&E stain) (X400)

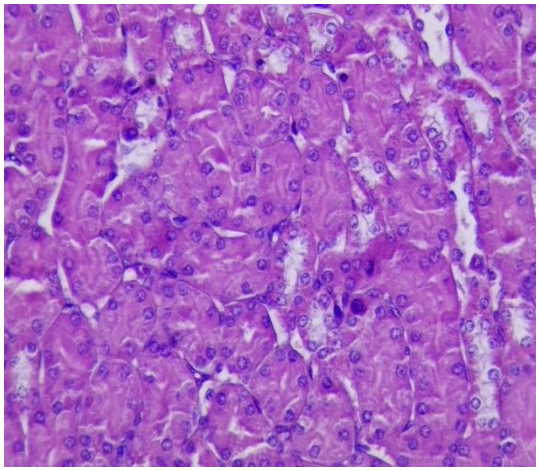

Figure 3: Histological sections in mice's kidneys treated with Sildenafil (2.5 mg/kg) showed kidney tissue free from necrosis and inflammatory cells (H&E stain) (X400)

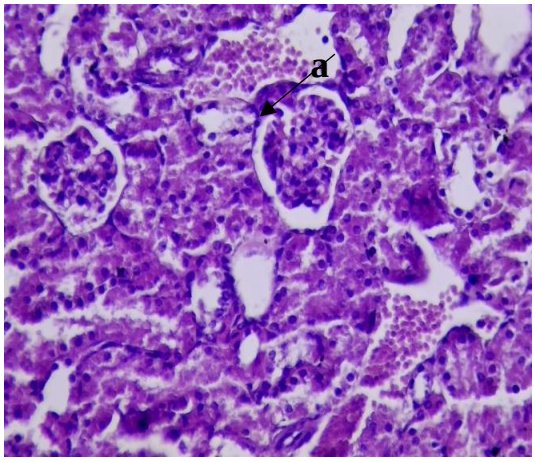

Figure 4: Histological sections in the kidneys of mice treated with Sildenafil (0.5 mg/kg) showed a: necrosis (H&E stain) (X400)

Conclusion

Through its anti-inflammatory, antioxidant, and anti-apoptotic characteristics, sildenafil protects against the neurodegenerative effects of an adenine-induced CKD model.

Ethical clearance

All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the scientific research ethics committee of the University of Baghdad, College of Science, Department of Biology, under the number CSEC/0922/0078.

Conflict of interest

The author declares that they have no conflict of interest.

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