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Adropin as a protective agent against renal ischemia-reperfusion injury induced by suprarenal aortic cross-clamping in rats

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Abstract

Background The development of protective therapeutic strategies against acute kidney injury associated with suprarenal aneurysms, renal artery occlusive disease, and suprarenal aortic reconstruction is of paramount importance. Adropin is a peptide hormone that has been shown to protect vascular endothelial cells and reduce oxidative stress, apoptosis, and inflammation. Therefore, in addition to its metabolic and vascular effects, adropin has potential as a therapeutic agent in renal ischemia-reperfusion injury. This study aims to investigate the protective effects of adropine on kidney ischemia-reperfusion (IR) injury under the suprarenal aortic cross clamp.

Methods Male Sprague Dawley rats were divided into six groups, with seven rats in each group for the study design. The control and ischemia reperfusion (IR) induced groups were designated as the two groups while the other four groups (TR1 to TR4) were administered varying doses of adropin at 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, and 2 mg/kg for each group. After a 60 min ischemic period, a 24-hour reperfusion period was implemented to assess the outcomes of adropin treatment on renal IR. Histopathological analysis was performed in conjunction with determination of apoptosis, and malondialdehyde (MDA) levels. In addition, serum concentrations of adropin, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), as well as endothelial nitric oxide synthase (eNOS) were measured in order to further define the biochemical reactions of the treatment.

Results MDA levels were significantly elevated in the IR group compared to the control group, while the activities of eNOS, SOD, and GSH-Px enzymes were significantly decreased ($P < 0.05$). MDA levels in the treatment groups were lower than those in the IR group, whereas eNOS, SOD, and GSH-Px levels were higher ($P < 0.05$). Statistically, the lowest adropin levels were observed in the IR group, while the highest levels were noted in the TR4 group ($P < 0.05$). Histopathological examination revealed a reduction in tissue damage in the treatment groups compared to the IR group.

Conclusion The histological and biochemical findings from this study indicate that adropin provides protective effects against renal ischemia-reperfusion injury in a dose-dependent manner.

Keywords Adropin, Apoptosis, Inflammation, Renal ischemia/Reperfusion injury, Oxidative stress

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Introduction

Acute kidney injury (AKI) is a common pathological condition that can lead to chronic kidney disease. One of the primary causes of AKI is ischemia-reperfusion (IR) damage [1]. Ischemia initiates a series of biochemical reactions that lead to cellular dysfunction and eventual cell death [2]. However, the restoration of oxygen to ischemic tissue can exacerbate kidney damage through oxidative stress [3, 4]. During kidney IR damage, levels of blood and tissue oxidant indicators increase, while antioxidant levels decrease. As a result, microvascular disorders, inflammation, necroptosis, and apoptosis of tubular epithelial cells can occur, leading to homeostatic imbalances [5].

Discovered in 2008, adropin is a peptide hormone composed of 76 amino acids, with a similar sequence in humans, mice, and rats [6]. Adropin has been identified in various tissues and body fluids, including the brain, cerebellum, liver, kidney, heart, pancreas, small intestine, endothelial cells, colostrum, and other body fluids [7]. Studies reveal that adropin regulates energy homeostasis, protects against endothelial dysfunction, and plays a role in insulin resistance and cell communication [8]. It has been shown that adropin levels can decrease in various inflammatory metabolic diseases; however, there is limited data on the relationship between adropin and kidney diseases [9].

Undoubtedly, the kidneys are among the most affected organs during the cross-clamping process in classical suprarenal aortic surgery. In cases of abdominal aortic diseases, even when the pathology is located at the infrarenal level, the surgeon may need to apply the cross-clamp at the suprarenal level [10]. Ischemia and reperfusion cause changes in the kidney vascular structure and endothelial function in acute kidney injury [11]. Therefore, agents involved in NO-dependent signaling pathways may play a role in kidney ischemia-reperfusion injury. Adropin, which upregulates eNOS protein levels in endothelial cells, has also demonstrated anti-apoptotic, anti-oxidative, and anti-inflammatory effects in both in vitro and in vivo studies [12–14]. Therefore, the primary aim of this study is to investigate the protective effect of different doses of exogenous adropin against renal IR injury caused by a cross-clamp placed in the suprarenal aorta. We believe that the potential positive outcomes of this study may provide a safer surgical environment for surgeons performing suprarenal aortic procedures.

Materials and methods

Animals

This study was approved by the Adiyaman University Animal Experiments Local Ethics Committee (Approval No: 2019/37). Male Sprague-Dawley rats weighing 250–280 g and aged 10–12 weeks, obtained from the Adiyaman University Experimental Animal Production and Research Centre, were divided into six groups, each containing seven animals. The experiments were carried out according to ARRIVE guidelines and the US National Institutes of Health, with the approval of the Research and Ethics Committee of Adıyaman University of medical sciences. All animals were not treated for the first 7 days to facilitate cage adaptation. They were housed at a room temperature of 22 °C ± 2 °C, with alternating 12-hour light and dark cycles, and were provided with food and water ad libitum during both the adaptation and experimental periods.

Experimental design and surgical procedures

Control group (n: 7)

The animals were anesthetized using intraperitoneal (i.p.) injections of 50 mg/kg ketamine and 10 mg/kg xylazine. Following laparotomy, the intestines were retracted to access the suprarenal aorta, and the adipose tissue surrounding the aorta was cleared. No further procedures were performed, and the abdominal incision was sutured according to the bilayer abdominal incision model. Sterile saline (SS) was administered at 5% of body weight to replace the fluid lost during the abdominal opening. After 24 h, blood and tissue samples were collected under anesthesia, and the animals were subsequently decapitated (Table 1).

IR group (n: 7)

The animals were anesthetized using intraperitoneal (i.p.) injections of 50 mg/kg ketamine and 10 mg/kg xylazine. Following laparotomy, the intestines were retracted to access the suprarenal aorta, and the adipose tissue surrounding the aorta was cleared before applying the clamp. Appropriate induction of ischemia was confirmed based on the intensity of color fading in the tissues within a few minutes of clamping. After 60 min of ischemia, the clamp was removed to initiate reperfusion. The abdominal incision was then sutured according to the two-layer incision model. Sterile saline was administered at 5% of body weight to replace the fluids lost during the abdominal opening. After 24 h of reperfusion, blood and tissue samples were collected under anesthesia, and the animals were subsequently decapitated (Table 1).

Treatment groups (TR1;TR2;TR3;TR4)

The animals in these four groups were administered adropin at the following doses: 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, and 2 mg/kg, via intraperitoneal (i.p.) injection 30 min before the surgical procedures and arterial clamping. All subsequent procedures were conducted in accordance with those in the IR group (Table 1).

We performed cervical dislocation as a humane euthanasia method for rats, ensuring that each animal was

Table 1 Description of the experimental groups

Group	Anesthesia Method	Surgical Procedure	Treatment	Ischemic Procedure	Reperfusion Time
Control Group (*N* = 7)	50 mg/kg ketamine + 10 mg/kg xylazine (i.p.)	Laparotomy, bilayer abdominal incision model	Sterile saline (5% body weight) 30 min before the surgical procedures	-	24 h reperfusion
IR Group (*N* = 7)	50 mg/kg ketamine + 10 mg/kg xylazine (i.p.)	Laparotomy, suprarenal aorta clamping, bilayer abdominal incision model	Sterile saline (5% body weight) 30 min before the surgical procedures	60 min of ischemia	24 h reperfusion
Treatment Group 1 (TR1) (*N* = 7)	50 mg/kg ketamine + 10 mg/kg xylazine (i.p.)	Laparotomy, suprarenal aorta clamping, bilayer abdominal incision model	0.5 mg/kg Adropin (i.p.) application 30 min before the surgical procedures	60 min of ischemia	24 h reperfusion
Treatment Group 2 (TR2) (*N* = 7)	50 mg/kg ketamine + 10 mg/kg xylazine (i.p.)	Laparotomy, suprarenal aorta clamping, bilayer abdominal incision model	1 mg/kg Adropin (i.p.) 30 min before the surgical procedures	60 min of ischemia	24 h reperfusion
Treatment Group 3 (TR3) (*N* = 7)	50 mg/kg ketamine + 10 mg/kg xylazine (i.p.)	Laparotomy, suprarenal aorta clamping, bilayer abdominal incision model	1.5 mg/kg Adropin (i.p.) 30 min before the surgical procedures	60 min of ischemia	24 h reperfusion

fully anesthetized prior to the procedure. This technique was carried out by researchers to minimize distress and adhered to our institution's ethical guidelines for animal care. Blood samples from all animals were centrifuged, and the resulting serum samples were stored at − 20 °C until the enzyme-linked immunosorbent assay (ELISA) was performed.

Biochemical analysis

Measurement of SOD and GSH-Px activities in serum

GSH-Px and SOD enzyme activities were determined using the rat GSH-Px ELISA Kit (Rel Assay Diagnostics, Gaziantep, Turkey, LOT No: 201-11-1705) and the rat SOD ELISA Kit (Rel Assay Diagnostics, LOT No: 201-11-1697). Measurements were performed according to the manufacturer's instructions and read on an ELISA reader (Bio-Tek ELX800 ELISA, BioTek Instruments, USA) at 450 nm.

Measurement of MDA level in kidney tissue

Measurement of MDA levels in kidney tissue was performed as follows: First, kidney tissue samples were homogenized using a buffer consisting of 14.5 mmol Tris base, 36.5 mmol Tris-HCl, 161 mmol KCl, and 2% Tween 20. Tissue samples were weighed, and 5 ml of the buffer was added. The mixture was then homogenized at 16,000 rpm for 3 min using an Ultra Turrax Type T25-B homogenizer (IKA Labortechnic, Germany). The homogenates were centrifuged at 5,000 rpm for 5 min. Subsequently, 1 ml of the supernatant was mixed with 1 ml of 10% (w/v) trichloroacetic acid (TCA), 1 ml of 0.6% (w/v) thiobarbituric acid, 1 ml of distilled water, and 0.5 ml of 4% (v/v) hydrochloric acid. This mixture was incubated at 95 °C for 120 min and then allowed to cool to room temperature. Finally, 3 ml of butanol was added to each tube, and the mixture was vortexed and centrifuged at 5,000 rpm for 5 min. The butanol phase was collected, and absorbance was measured at 532 nm, using butanol as the blank. The results were expressed as nanomoles per gram of tissue.

Measurement of adropin and eNOS activities in serum

Adropin and eNOS enzyme activities were determined using the rat adropin ELISA kit (Rel Assay Diagnostics, LOT No: 201-11-1717) and the rat eNOS ELISA kit (SunRed Biotechnology Company, Shanghai, China, LOT No: 201-11-0466). Measurements were conducted according to the manufacturer's instructions and were read on an ELISA reader (Bio-Tek ELX800, BioTek Instruments, USA) at 450 nm. The results were expressed as ng/ml.

Histopathological analysis

After the rats were decapitated, kidneys were removed, weighed, and rapidly fixed in 10% buffered formalin. After routine tissue processing, 5-µm thick tissue sections were obtained from the paraffin blocks, stained with hematoxylin-eosin (H&E), and evaluated using with light microscopy (Leica DM500 attached Leica DFC295 Digital Image Analyze System).

Apoptotic analysis

Investigation of apoptosis was performed through the immunohistochemical determination of caspase-3 positivity in kidney tissue. For this, 5-µm sections obtained from paraffin blocks were placed on poly-L-lysine slides. The deparaffinized tissues were passed through a graded alcohol series and then incubated in citrate buffer solution at pH 6 in a microwave oven (750 W) for a total of 12 min (7 min followed by an additional 5 min) to retrieve the antigen. Sections were treated with Ultra V Block solution to prevent background staining. After this, the sections were incubated with caspase-3 antibody (Caspase-3, Rabbit polyclonal IgG, ab2302, Abcam, London,

UK) for 60 min. Following this, they were treated with a secondary antibody for 30 min, streptavidin alkaline phosphatase for 30 min, and then the Fast Red Substrate System. Tissues were counterstained with Mayer's hematoxylin, washed with phosphate-buffered saline and distilled water, and then covered with a coverslip using aqua medium. The prepared slides were examined under a light microscope and photographed using a Leica DM500 attached to a Leica DFC295 Digital Image Analysis System. Histoscores were based on the prevalence interval for immunoreactivity as follows: <25%, 0.1; 26–50%, 0.4; 51–75%, 0.6; and 76–100%, 0.9. Staining intensity was classified as absent (0), very low (+0.5), low (+1), moderate (+2), and severe (+3). The histopathological score was calculated as the prevalence multiplied by the staining intensity.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism 8 software. Firstly normalization analysis done for all tests. The One-way Anova was used for intergroup comparisons of MDA, SOD, GSH-Px, eNOS, and immunity variables. Tukey's pairwise multiple comparison test was used to determine the intergroup differences between the significant variables.

Results

Serum SOD levels

The SOD values of the control group did not show a significant difference when compared to those of the TR1 and TR2 groups; however, they were significantly higher than those of the other groups ($P<0.05$). The SOD levels in the TR1, TR2, and TR3 groups were similar and significantly higher than those in the IR and TR4 groups ($P<0.001$) (Fig. 1).

Serum GSH-Px levels

GSH-Px levels were found to be lower in the IR group compared to the control group ($p=0.0054$). After treatment, GSH-Px levels increased; however, they displayed an inverse response to the increase in dose. Notably, the lowest dose exhibited the highest efficacy (Fig. 2).

MDA levels in kidney tissue

MDA values in the IR group were significantly higher than those in the other groups ($p<0.0001$). There were no significant differences in MDA levels between the TR1 and TR2 groups compared to the control group; however, the TR3 and TR4 groups exhibited significantly elevated MDA levels ($p<0.0001$) (Fig. 3).

Serum Adropin levels

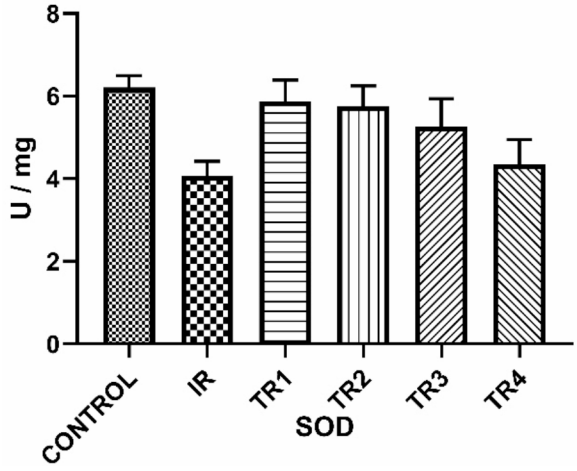
Adropin levels were observed to be significantly lower in the IR group ($P<0.0001$) and, as expected, increased after treatment in a dose-dependent manner (Fig. 4). Administration of 1.5 mg/kg ($p=0.0002$) and 2 mg/kg ($P<0.0001$) of adropin resulted in a significant increase compared to the control group.

Serum eNOS levels

Serum eNOS levels in the control group were found to be significantly higher than those in the IR group ($P<0.0001$) and the TR4 group ($P=0.0013$). Statistically significant increases were observed in the TR1 ($P=0.0083$) and TR3 ($P=0.0318$) groups compared to the IR group (Fig. 5).

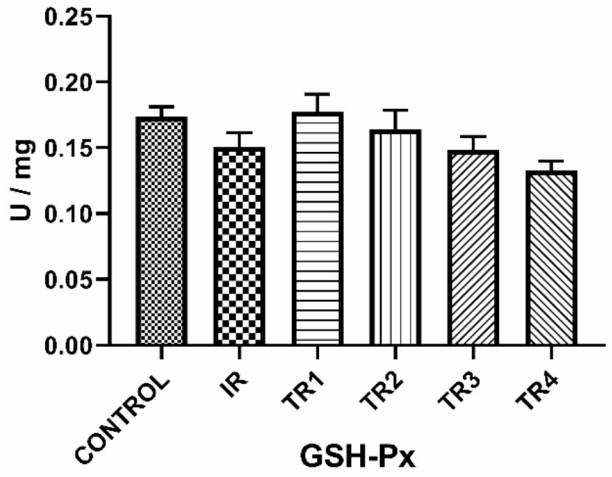
Effect of adropin on kidney histopathology

Histopathological examination revealed no changes in the kidney tissue of the control group, which underwent sham surgery without ischemia (Fig. 6a). In contrast, severe kidney tissue damage was observed in the IR



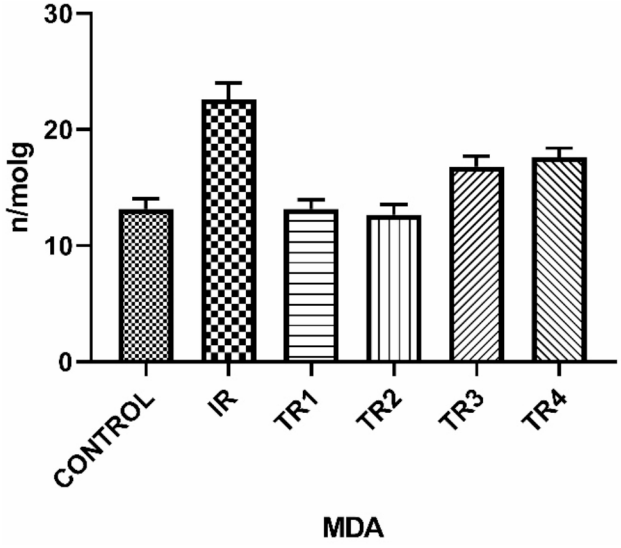
Tukey's multiple comparisons test	Mean Diff.	Summary	Adjusted P Value
CONTROL vs. IR	2,137	****	<0,0001
CONTROL vs. TR1	0,3414	ns	0,8011
CONTROL vs. TR2	0,4543	ns	0,5505
CONTROL vs. TR3	0,9571	*	0,0130
CONTROL vs. TR4	1,854	****	<0,0001
IR vs. TR1	-1,796	****	<0,0001
IR vs. TR2	-1,683	****	<0,0001
IR vs. TR3	-1,180	**	0,0013
IR vs. TR4	-0,2829	ns	0,8979

Fig. 1 Effects of different doses of adropin administration on SOD levels in ischemia reperfusion injury (Following the ischemia-reperfusion (IR) application, a significant reduction in superoxide dismutase (SOD) levels was observed ($P<0.001$). Treatment with adropin resulted in a notable increase in SOD levels. The specific differences between the treatment groups are detailed in the accompanying table)



Tukey's multiple comparisons test	Mean Diff,	Summary	Adjusted P Value
CONTROL vs. IR	0,02257	**	0,0054
CONTROL vs. TR1	-0,004000	ns	0,9824
CONTROL vs. TR2	0,009714	ns	0,5616
CONTROL vs. TR3	0,02543	**	0,0013
CONTROL vs. TR4	0,04071	****	<0,0001
IR vs. TR1	-0,02657	***	0,0008
IR vs. TR2	-0,01286	ns	0,2601
IR vs. TR3	0,002857	ns	0,9962
IR vs. TR4	0,01814	*	0,0390

Fig. 2 Effects of different doses of adropin administration on GSH-Px levels in ischemia reperfusion injury. (GSH-Px levels in the ischemia-reperfusion (IR) group were significantly lower compared to the control group ($P = 0.0054$). The TR1 group exhibited significantly higher GSH-Px levels than the IR group ($P = 0.0008$), while the TR4 group had significantly lower GSH-Px levels ($P = 0.0390$). The specific differences between the treatment groups are detailed in the accompanying table)



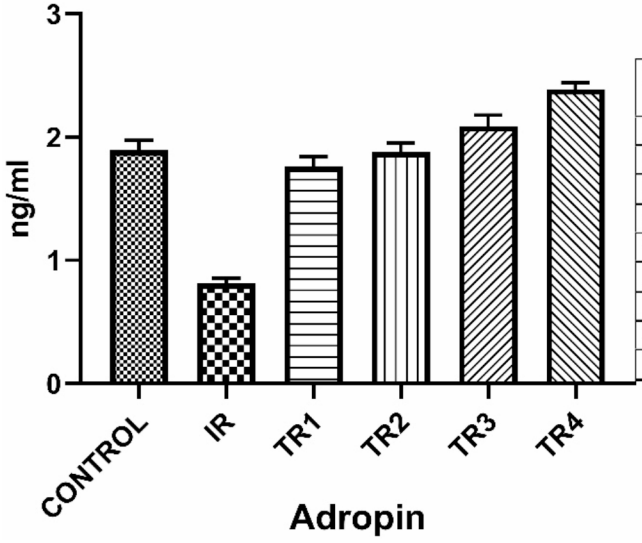
Tukey's multiple comparisons test	Mean Diff,	Summary	Adjusted P Value
CONTROL vs. IR	-9,434	****	<0,0001
CONTROL vs. TR1	0,01429	ns	>0,9999
CONTROL vs. TR2	0,5086	ns	0,9154
CONTROL vs. TR3	-3,651	****	<0,0001
CONTROL vs. TR4	-4,451	****	<0,0001
IR vs. TR1	9,449	****	<0,0001
IR vs. TR2	9,943	****	<0,0001
IR vs. TR3	5,783	****	<0,0001
IR vs. TR4	4,983	****	<0,0001

Fig. 3 Effects of different doses of adropin administration on MDA levels in ischemia reperfusion injury. (Malondialdehyde (MDA) levels were significantly elevated in the ischemia-reperfusion (IR) group compared to the other groups ($P < 0.0001$). Additionally, the TR3 and TR4 groups showed significantly higher MDA levels than the control group ($P < 0.0001$). No significant differences in MDA levels were observed between the TR1 and TR2 groups compared to the control group. The specific differences between the treatment groups are detailed in the accompanying table)

group (Fig. 6b). This injury was characterized by congestion, bleeding, tubular necrosis, tubular dilatation, and the presence of protein casts within the tubules. Administration of 0.5 mg/kg, 1 mg/kg, and 1.5 mg/kg of adropin significantly reduced the extent of kidney tissue injuries (Fig. 6c and d, and 6e, respectively). However, administration of 2 mg/kg of adropin did not show any protective effect against kidney damage (Fig. 6f).

Immunoreactivity of apoptosis

Caspase-3 positive cells were identified in the tubular, glomerular, and interstitial areas (Fig. 7a-f). Histoscores were calculated, allowing for comparison among all groups relative to the IR group. Significant differences were observed in the control ($p = 0.0198$), TR1 ($p = 0.0131$), and TR2 ($p = 0.0198$) groups (Fig. 8).



Tukey's multiple comparisons test	Mean Diff,	Summary	Adjusted P Value
CONTROL vs. IR	1,081	****	<0,0001
CONTROL vs. TR1	0,1357	*	0,0140
CONTROL vs. TR2	0,01571	ns	0,9984
CONTROL vs. TR3	-0,1943	***	0,0002
CONTROL vs. TR4	-0,4929	****	<0,0001
IR vs. TR1	-0,9450	****	<0,0001
IR vs. TR2	-1,065	****	<0,0001
IR vs. TR3	-1,275	****	<0,0001
IR vs. TR4	-1,574	****	<0,0001

Fig. 4 The levels of recombinant adropin administration against ischemia reperfusion injury in all groups (Adropin levels were significantly lower in rats with ischemia-reperfusion (IR) compared to the control group ($P < 0.0001$). Notably, following treatment, adropin levels increased in a dose-dependent manner. The specific differences between the treatment groups are detailed in the accompanying table)

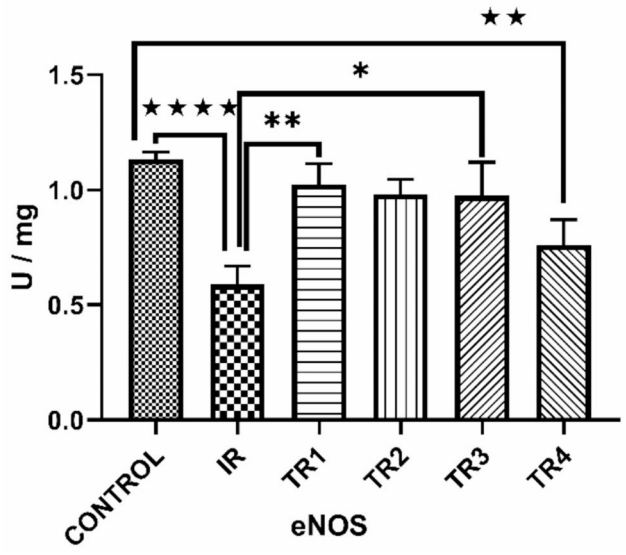


Fig. 5 The effects of adropin on eNOSlevels in serum samples of all groups. eNOS levels were significantly lower in the ischemia-reperfusion (IR) group compared to the control group ($P < 0.0001$). Following treatment, both the TR1 ($P = 0.0083$) and TR3 ($P = 0.0318$) groups exhibited significantly increased eNOS levels compared to the IR group

Discussion

Our study demonstrated that adropin attenuates renal IR injury by regulating eNOS, markers of oxidative stress (MDA, SOD, GSH-Px), and apoptosis. Importantly, the doses at which adropin produces its actions are in a critical nature, as lower doses demonstrate beneficial effects, while ever-higher doses fail to show improvement, or worse, may even prove harmful.

Adropin is associated with various metabolic risk factors. Plasma adropin levels are negatively correlated with obesity and insulin resistance [15]. Low serum adropin levels have been observed in patients with cardiac X syndrome and severe atherosclerosis [16, 17]. Furthermore, adropin has been shown to reduce endothelial cell permeability and modulate ischemia-induced blood-brain barrier injury [18]. In addition to its metabolic properties, adropin also exhibits non-metabolic effects. It has been revealed to improve perfusion and increase capillary density in hindlimb ischemia [19]. Although data on adropin levels in kidney disease is limited, a negative correlation has been noted between this peptide and the progression of kidney failure. In our study, adropin levels were found to be significantly lower in the IR group, suggesting that adropin plays a role during the ischemia-reperfusion process and may be beneficial for patients with acute kidney injury (AKI).

Adropin regulates endothelial function by upregulating endothelial nitric oxide synthase (eNOS), which is a major component of the reperfusion injury salvage kinase (RISK) pathway and a crucial survival mechanism against ischemia-reperfusion (I/R) injury [19, 20]. In our study, we observed a significant increase in eNOS levels following treatment with adropin, indicating its protective effect.

During the ischemia-reperfusion procedure, blood flow is temporarily interrupted in the ischemic tissue. However, the subsequent restoration of blood flow paradoxically increases tissue damage [21]. Additionally, the reintroduction of oxygen to ischemic tissue exacerbates kidney damage through oxidative stress and lipid peroxidation. Malondialdehyde (MDA) is an excellent indicator of lipid peroxidation. Excessive lipid peroxidation during the acute phase after reperfusion, along with

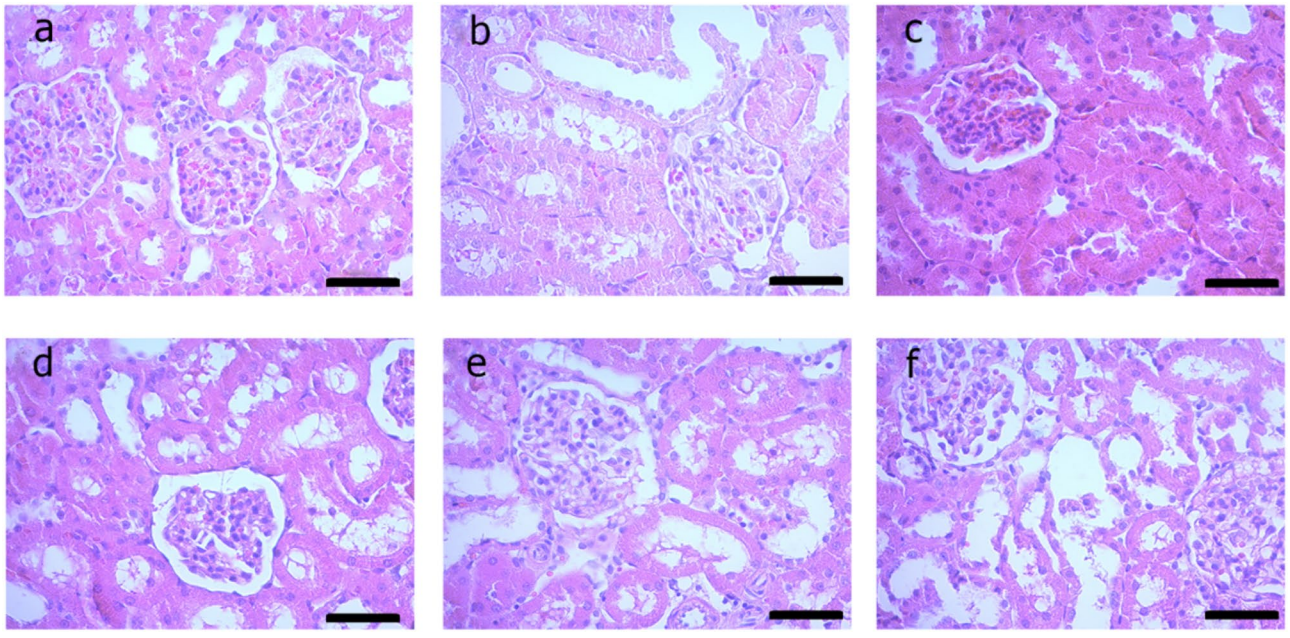


Fig. 6 In ischemia reperfusion injury, the effects of different doses of adropin administration on histopathological changes in rat kidney tissue in the experimental groups are observed; Accordingly histopathological examination revealed that the control group exhibited normal histological appearance (**a**) In contrast, the IR group demonstrated marked glomerular atrophy and tubular dilatation compared to the control group (**b**) A significant reduction in tubular dilatation and glomerular atrophy was observed in the TR1 (**c**), TR2 (**d**), and TR3 (**e**) groups relative to the IR group. However, the TR4 (**f**) group exhibited higher levels of damage compared to the IR group. (Hematoxylin eosin staining, Scala bar 50 µm)

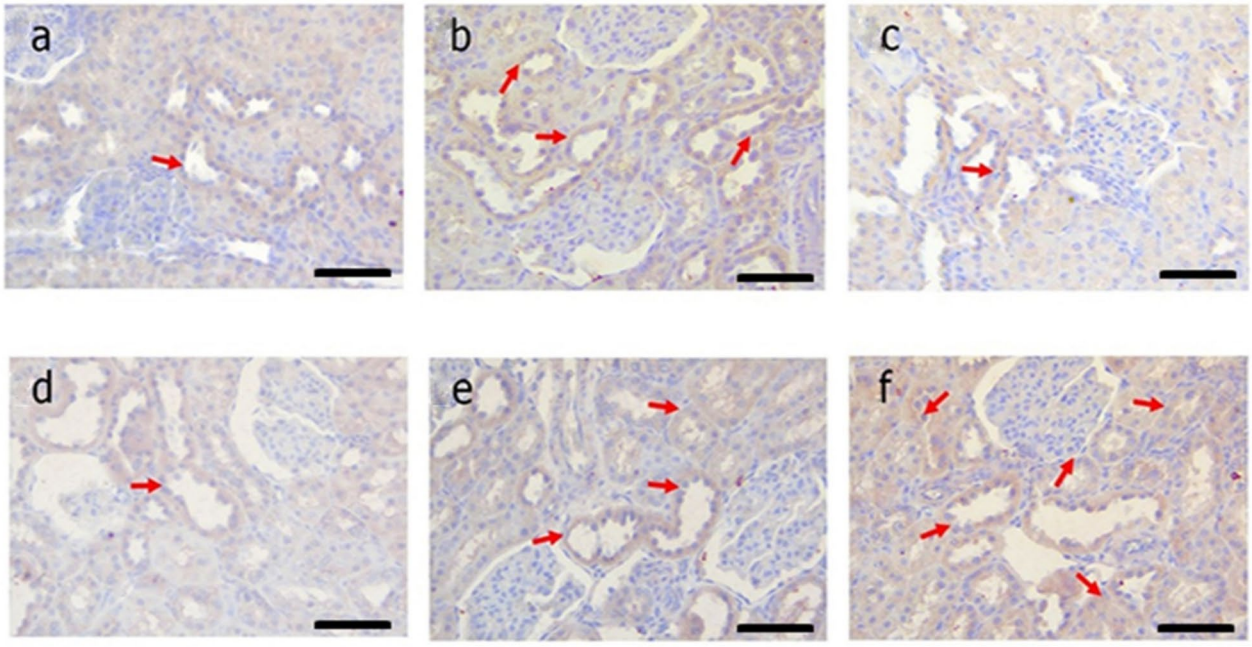


Fig. 7 Caspase-3 immunoreactivity in rat kidney tissue of all groups is demonstrated with red arrow. (Caspase-3 immunoreactivity was similar in TR1 and TR2 groups in which different doses of adropin were given compared to the control group. however, caspase-3 immunoreactivity was increased in the IR group compared to the control, TR1, TR2 groups. however, caspase-3 immunoreactivity was increased in the increasing adropin dose groups (TR3, TR4 groups) given with reperfusion after ischemic injury compared to the IR group. Control (**a**), IR (**b**), TR1 (**c**), TR2 (**d**), TR3 (**e**), and TR4 (**f**) AEC chromogen, Mayers Hematoxylin scala bar: 50 µm)

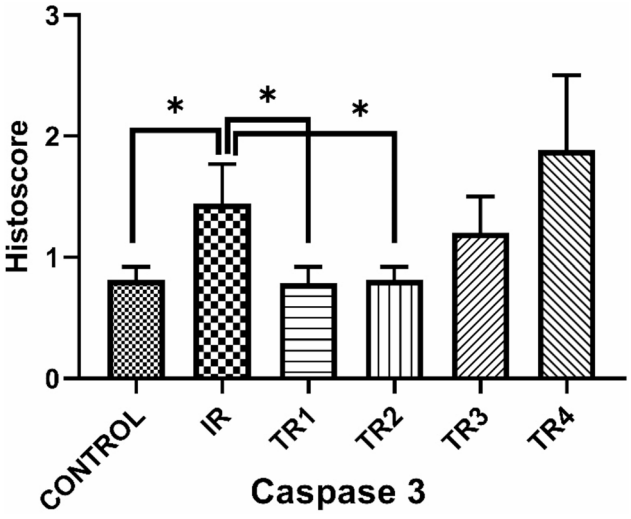


Fig. 8 Histoscoring of Apoptotic Immunoreactivity. Apoptotic immunoreactivity levels in the TR1 ($p = 0.0131$), TR2 ($p = 0.0198$), and Control groups ($p = 0.0198$) were significantly lower than those in the IR group ($p = 0.0392$)

alterations in other antioxidant processes, contributes to tissue damage [22]. Previous IR studies have shown that MDA levels increase in blood and tissues while superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels decrease, a finding supported by our study [23–25]. Notably, administration of 0.5 and 1 mg/kg of adropin resulted in increased SOD and GSH-Px enzyme activity and decreased MDA levels. However, contrary to expectations, no effects were observed in the groups that received 1.5 and 2 mg/kg of adropin.

eNOS is one of the vasoactive isoenzymes responsible for the production of nitric oxide [26]. It is expressed in endothelial cells and plays a crucial role in regulating blood flow, particularly in conditions that disrupt normal blood flow [27]. Several studies on ischemia-reperfusion (IR) injury have highlighted the beneficial effects of eNOS [28, 29], while the deleterious effects of inducible nitric oxide synthase (iNOS), another isoform of nitric oxide synthase, have also been reported [30, 31]. Notably, adropin positively influences endothelial function and enhances eNOS activity [32]; however, it also affects iNOS expression [33], In our study, a significant increase in eNOS levels was observed in the groups administered adropin, excluding the 2 mg/kg group, compared to the IR group.

During the ischemia-reperfusion process, various vascular lesions, including endothelial damage, congestion, and edema, occur alongside cascade reactions such as inflammatory cell infiltration, degeneration, necrosis, and fibrosis in the kidneys [5, 34]. A study examining IR-related kidney damage noted findings such as desquamation, tubular dilation, and hydropic changes in the tubular epithelium [35]. Our study observed similar pathological changes but also reported a significant improvement in renal morphology in the adropin-treated groups (except for the 2 mg/kg group) compared to the IR group.

Upregulation of caspase-3, a key effector of apoptosis, has been documented in renal tubular and microvascular endothelial cells during the early stages of IR-induced kidney injury [36, 37]. Additionally, caspase-3 deficiency has been shown to exacerbate necroptosis and increase susceptibility to tubular injury during the initial phase of IR-induced kidney damage [38]. Notably, studies have indicated that inhibiting caspase-related apoptotic pathways can enhance organ survival [39, 40]. In our findings, the application of low doses of adropin exhibited a protective effect, while higher doses correlated with increased cell death. This suggests that adropin may confer benefits in acute kidney injuries in a dose-dependent manner.

There are several potential limitations on this study. Although a range of doses were investigated for adropin in the study, the higher doses of 1.5 mg/kg and 2 mg/kg showed no added benefit. Further mechanistic studies to investigate why higher doses of adropin may lead to detrimental effects could help optimize dosing strategies for kidney.In addition to, further exploration of the pharmacokinetics and pharmacodynamics of adropin is warranted. Although the apoptotic marker caspase 3, eNOS and markers of oxidative stress were investigated, other potential signaling pathways or molecular mechanisms through which adropin exerts its effects-such as autophagy, inflammasome activation, or other nitric oxide-related pathways-were not deeply explored. While histological examination gives insight into renal damage, functional assessment, such as serum creatinine levels and glomerular filtration rate, would complement the findings and give a clearer picture of the recovery of kidney function after treatment with adropin. Finally, it would be fruitful to consider future research directions, such as the effects of adropin in specific models of chronic kidney disease or in conjunction with other drugs.

Conclusion

This study revealed that adropin reduces renal IR damage in a dose-dependent manner by preserving the renal histology, regulating eNOS activity and maintaining the oxidant/antioxidant balance. We believe that adropin is worth investigating, particularly for aortic aneurysm surgeries involving the renal artery (those with suprarenal aortic clamps). Therefore, ascertaining the protective effects of adropin will provide surgeons with an insight into safer clamp times.

Abbreviations

IR	Ischemia-reperfusion
MDA	Malondialdehyde
SOD	Superoxide Dismutase

eNOS	Endothelial nitric oxide synthase
iNOS	Inducible nitric oxide synthase
GSH-Px	Glutathione Peroxidase
AKI	Acute kidney injury
H&E	Hematoxylin and eosin staining
RISK	Reperfusion injury salvage kinase

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Data availability
The datasets generated and/or analysed during the current study are not publicly available due to privacy concerns but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Adiyaman University Animal Experiments Local Ethics Committee (Approval No: 2019/37).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. J Clin Investig. 2011;121(11):4210–21.
2. Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol Cell Physiol. 2002;282(2):C227–41.
3. Ratliff BB, Abdulmahdi W, Pawar R, Wolin MS. Oxidant mechanisms in renal injury and disease. Antioxid Redox Signal. 2016;25(3):119–46.
4. Wang ZH, Deng LH, Chi CW, Wang H, Huang YY, Zheng Q. A preclinical systematic review of Curcumin for protecting the kidney with ischemia reperfusion injury. Oxid Med Cell Longev. 2020;2020:4546851.
5. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest. 2011;121(11):4210–21.
6. Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN, et al. Identification of Adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metab. 2008;8(6):468–81.
7. Marczuk N, Cecerska-Heryć E, Jesionowska A, Dołęgowska B. Adropin - physiological and pathophysiological role. Postepy Hig Med Dosw (Online). 2016;70(0):981–8.
8. Maciorkowska M, Musiałowska D, Małyszko J. Adropin and Irisin in arterial hypertension, diabetes mellitus and chronic kidney disease. Adv Clin Exp Med. 2019;28(11):1571–5.
9. Zhang S, Chen Q, Lin X, Chen M, Liu Q. A review of Adropin as the medium of dialogue between energy regulation and immune regulation. Oxid Med Cell Longev. 2020;2020:3947806.
10. Varkevisser RRB, O'Donnell TFX, Swerdlow NJ, Liang P, Li C, Ultee KHJ, et al. Fenestrated endovascular aneurysm repair is associated with lower perioperative morbidity and mortality compared with open repair for complex abdominal aortic aneurysms. J Vasc Surg. 2019;69(6):1670–8.
11. Basile P, Yoder DC. Renal endothelial dysfunction in acute kidney ischemia reperfusion injury. Cardiovasc Haematol Disorders-Drug Targetsrug Targets-Cardiovascular Hematol Disorders). 2014;14(1):3–14.
12. Wu L, Fang J, Yuan X, Xiong C, Chen L. Adropin reduces hypoxia/reoxygenation–induced myocardial injury via the reperfusion injury salvage kinase pathway. Experimental Therapeutic Med. 2019;18(5):3307–14.
13. Li B, Wang Z, He Y, Chen T, Zhang Y, Yuan X, et al. Adropin improves Radiation-Induced myocardial injury via VEGFR2/PI3K/Akt pathway. Oxidative Med Cell Longev. 2022;2022(1):8230214.
14. Chen L, Lu J, Hu J, Gong X. Unveiling the multifaceted role of Adropin in various diseases. Int J Mol Med. 2024;54(4):90.
15. Butler AA, Tam CS, Stanhope KL, Wolfe BM, Ali MR, O'Keeffe M, et al. Low Circulating Adropin concentrations with obesity and aging correlate with risk factors for metabolic disease and increase after gastric bypass surgery in humans. J Clin Endocrinol Metab. 2012;97(10):3783–91.
16. Celik A, Balin M, Kobat MA, Erdem K, Baydas A, Bulut M, et al. Deficiency of a new protein associated with cardiac syndrome X; called Adropin. Cardiovasc Ther. 2013;31(3):174–8.
17. Wu L, Fang J, Chen L, Zhao Z, Luo Y, Lin C, et al. Low serum Adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. Clin Chem Lab Med. 2014;52(5):751–8.
18. Yang C, DeMars KM, Hawkins KE, Candelario-Jalil E. Adropin reduces paracellular permeability of rat brain endothelial cells exposed to ischemia-like conditions. Peptides. 2016;81:29–37.
19. Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta M, et al. Adropin is a novel regulator of endothelial function. Circulation. 2010;122(11 Suppl):S185–92.
20. Hu W, Chen L. Association of serum Adropin concentrations with diabetic nephropathy. Mediators Inflamm. 2016;2016:6038261.
21. Koo DD, Welsh KI, West NE, Channon KM, Penington AJ, Roake JA, et al. Endothelial cell protection against ischemia/reperfusion injury by lecithinized superoxide dismutase. Kidney Int. 2001;60(2):786–96.
22. Kadenbach B, Ramzan R, Moosdorf R, Vogt S. The role of mitochondrial membrane potential in ischemic heart failure. Mitochondrion. 2011;11(5):700–6.
23. Scaduto RC Jr., Gattone VH 2nd, Martin LF, Yang HC. Elevation of renal glutathione enhances ischemic injury. Ren Physiol Biochem. 1991;14(6):259–70.
24. Bircan B, Çakır M, Kırbağ S, Gül HF. Effect of Apelin hormone on renal ischemia/reperfusion induced oxidative damage in rats. Ren Fail. 2016;38(7):1122–8.
25. Ain QT, Haq SH, Alshammari A, Al-Mutlaq MA, Anjum MN. The systemic effect of PEG-nGO-induced oxidative stress in vivo in a rodent model. Beilstein J Nanotechnol. 2019;10:901–11.
26. Mount PF, Power DA. Nitric oxide in the kidney: functions and regulation of synthesis. Acta Physiol (Oxf). 2006;187(4):433–46.
27. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov. 2008;7(2):156–67.
28. Chang WL, Chung CH, Wu YC, Su MJ. The vascular and cardioprotective effects of liriodenine in ischemia-reperfusion injury via NO-dependent pathway. Nitric Oxide. 2004;11(4):307–15.
29. Hines IN, Harada H, Flores S, Gao B, McCord JM, Grisham MB. Endothelial nitric oxide synthase protects the post-ischemic liver: potential interactions with superoxide. Biomed Pharmacother. 2005;59(4):183–9.
30. Qi WN, Chen LE, Zhang L, Eu JP, Seaber AV, Urbaniak JR. Reperfusion injury in skeletal muscle is reduced in inducible nitric oxide synthase knockout mice. J Appl Physiol (1985). 2004;97(4):1323–8.
31. Miller JT, Turner CG, Otis JS, Sebeh Y, Hayat MJ, Quyyumi AA, et al. Inhibition of iNOS augments cutaneous endothelial NO-dependent vasodilation in

prehypertensive non-Hispanic Whites and in non-Hispanic Blacks. Am J Physiol Heart Circ Physiol. 2021;320(1):H190–9.
32. Sena CM, Pereira AM, Seiça R. Endothelial dysfunction - a major mediator of diabetic vascular disease. Biochim Biophys Acta. 2013;1832(12):2216–31.
33. Kuloglu T, Aydin S. Immunohistochemical expressions of Adropin and ınducible nitric oxide synthase in renal tissues of rats with streptozotocin-ınduced experimental diabetes. Biotech Histochem. 2014;89(2):104–10.
34. Molitoris BA, Sutton TA. Endothelial injury and dysfunction: role in the extension phase of acute renal failure. Kidney Int. 2004;66(2):496–9.
35. Cakir M, Duzova H, Baysal I, Gül CC, Kuşcu G, Kutluk F, et al. The effect of hypericum perforatum on kidney ischemia/reperfusion damage. Ren Fail. 2017;39(1):385–91.
36. Zhang X, Zheng X, Sun H, Feng B, Chen G, Vladau C, et al. Prevention of renal ischemic injury by Silencing the expression of renal caspase 3 and caspase 8. Transplantation. 2006;82(12):1728–32.
37. Hörbelt M, Lee SY, Mang HE, Knipe NL, Sado Y, Kribben A, et al. Acute and chronic microvascular alterations in a mouse model of ischemic acute kidney injury. Am J Physiol Ren Physiol. 2007;293(3):F688–95.
38. Yang B, Lan S, Dieudé M, Sabo-Vatasescu JP, Karakeussian-Rimbaud A, Turgeon J, et al. Caspase-3 is a pivotal regulator of microvascular rarefaction and renal fibrosis after ischemia-Reperfusion injury. J Am Soc Nephrol. 2018;29(7):1900–16.
39. Ying J, Wu J, Zhang Y, Han Y, Qian X, Yang Q, et al. Ligustrazine suppresses renal NMDAR1 and caspase-3 expressions in a mouse model of sepsis-associated acute kidney injury. Mol Cell Biochem. 2020;464(1–2):73–81.
40. He Y, Lang X, Cheng D, Zhang T, Yang Z, Xiong R. miR–30a–5p inhibits hypoxia/reoxygenation–induced oxidative stress and apoptosis in HK–2 renal tubular epithelial cells by targeting glutamate dehydrogenase 1 (GLUD1). Oncol Rep. 2020;44(4):1539–49.

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