# The Effects of High Dose Sugammadex on Rat Kidney Tissue Following Unilateral Ureteral Obstruction

## Unilateral Üreter Obstrüksiyonunu Takiben Kullanılan Yüksek Doz Sugammadeks'in Sıçan Böbrek Dokusuna Etkisi

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**ABSTRACT**

**Background:** Unilateral ureteral obstruction (UUO) is commonly used model showed to imitate process of obstructive nephropathy in a feasible.
The aim of this study is to evaluate the histopathological and biochemical effects of high doses sugammadex on kidney tissue of the rats those have early and late phase renal failure induced by UUO.
**Materials and Methods:** Thirty male Wistar albino rats were randomly divided into five study groups. Control Group(C), UUO for 1 week Group(UUO-1), UUO-1 week treated with rocuronium and 96mg.kg$^{-1}$ Sugammadex Group(UUO-1 week-96S), UUO for 3 weeks Group(UUO-3) and UUO-3 weeks treated with rocuronium and 96mg.kg$^{-1}$ Sugammadex Group(UUO-3 week-96S). The blood samples are stored at -20 degrees and MDA and NO were studied in the serum. Kidney tissue was removed for histopathological examination.
**Results:** In the histopathological examination of all parameters (glomerular vacuolization(GV), tubular dilatation(TD), Vascular vacuolisation and hypertrophy(VVH), tubular cell degeneration and necrosis(TCDN), Bowman's space dilatation(BSD), tubular hyaline cylinders(THC), lymphocyte infiltration(LI), tubular cell sloughing(TCS), significant difference was observed for the kidney at the obstruction side. After 3 weeks, in the side of the all unobstructed groups' kidney tissues, significantly higher GV scores were detected compared with the GroupC. TD was observed more for the UUO1, UUO3 and UUO3S groups when compared with GroupC. And TCDN was observed more for the UUO1S and UUO3S groups when compared with GroupC.
When the groups were compared with each other; it is observed that MDA and NO enzyme activities of UUO1S, UUO3 and UUO3S groups were significantly difference GroupC.
**Conclusion:** High dose of sugammadex (96 mg.kg$^{-1}$) can be used safely in UUO cases, on the other hand significant attention should be paid in bilateral ureteral obstruction cases. Our findings may be a guide for future animal and human studies investigating the effects of sugammadex on kidney tissue.

**Key Words:** Unilateral ureteral obstruction, sugammadex, MDA, NO, kidney histopathology, rat

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**ÖZET**

**Amaç:** Unilateral üreter obstrüksiyonu (UÜO), ilerlemiş obstrüktif nefropatiyi taklit eden bir model olarak yaygınca kullanılmaktadır. Bu çalışmanın amacı, UÜO'nun neden olduğu erken ve geç dönem böbrek yetmezliği olan sıçanların böbrek dokusunda yüksek doz sugammadeks'in histopatolojik ve biyokimyasal etkilerini değerlendirmektir.
**Yöntem:** Otuz adet erkek Wistar albino sıçanı, randomize olarak, Kontrol grubu (K), 1 hafta süreyle UÜO grubu (UÜO-1), UÜO-1 hafta rokuronyum ve 96mg.kg$^{-1}$ sugammadeks ile tedavi edilen grup (UÜO-1 hafta-96S), 3 hafta süreyle UÜO grubu (UÜO-3) ve UÜO-3 hafta, rokuronyum ve 96mg.kg$^{-1}$ Sugammadeks (UÜO-3 hafta-96S) ile tedavi edilen grup olmak üzere beş çalışma grubuna ayrıldı. Kan örnekleri -20 derecede saklandı ve serumda MDA ve NO çalışıldı. Histopatolojik inceleme için böbrek dokusu çıkarıldı.
**Bulgular:** Tüm parametrelerin histopatolojik incelenmesinde glomerüler vakuolizasyon (GV), tübüler dilatasyon (TD), Vasküler vakuolizasyon ve hipertrofi (VVH), tübüler hücre dejenerasyonu ve nekrozu (THDN), Bowman boşluğu dilatasyonu (BBD), tübüler hiyalen silindirleri(THS), Lenfosit infiltrasyonu (Lİ), tübüler hücre yenilenmesi(THY) açısından obstrüksiyon tarafındaki böbrekde belirgin farklılık gözlendi. 3 hafta sonra, Grup K'yla karşılaştırıldığında obstrüksiyon olmayan grupların böbrek dokularının tamamında, anlamlı olarak daha yüksek GV skorları saptandı. TD, Grup K ile karşılaştırıldığında, UÜO1, UÜO3 ve UÜO3S gruplarında daha fazla gözlemlendi. THDN, Grup K ile karşılaştırıldığında UÜO1S ve UÜO3 S gruplarından daha fazla gözlemlendi.
Gruplar birbirleriyle karşılaştırıldığında; UÜO1S, UÜO3 ve UÜO3S gruplarının, MDA ve NO enzim aktiviteleri Group K'dan anlamlı farklı gözlemlendi.
**Sonuç:** UÜO'lu olgularda yüksek dozda sugammadeks (96 mg.kg$^{-1}$) güvenle kullanılabilirken, diğer taraftan bilateral üreteral obstrüksiyonlu olgularda önemle dikkat edilmelidir. Bulgularımız, sugammadeks's'in böbrek dokusu üzerindeki etkilerini araştıran gelecekteki hayvan ve insan çalışmaları için bir rehber olabilir.

**Anahtar Sözcükler:** Unilateral üreter obstrüuksiyonu, sugammadeks, MDA, NO, böbrek histopatolojisi, sıçan

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## INTRODUCTION

Obstructive nephropathy is one of the most common causes of renal insufficiency in children and adults. Also proportion of patients with known obstructive nephropathy –with or without renal failure- undergoing different surgical procedures under general anesthesia is nonignorable Unilateral ureteral obstruction (UUO) is commonly used model showed to imitate the process of obstructive nephropathy in a feasible, fast and species-independent manner(1,2). Peritubular capillaries rarefaction, tubular atrophy, inflammatory infiltration, widen interstitial space, and progressive tubulointerstitial fibrosis are important morphological features of obstructed kidneys(3,4). Compensatory hypertrophy of the contralateral kidney is a commonly encountered feature of UUO process(5).

In a study conducted by Hu et al, it was shown that weight of obstructed kidney is gradually declined 72 hours to 2 weeks after ligation. Also Peptides et al (6) showed that cortical thickness decreased from 24 hours to 3 months after ligation. On the other hand Dicker et al (5) showed that in order to compensate the damaged renal unit of the obstructed kidney, glomerular size of contralateral kidney gradually increased.

Following the obstruction several pathological changes in glomerular and particularly interstitial fields occur via several vasoactive factors and cytokines include prostaglandins (PG), angiotensin (ANG) II, growth factors and nitric oxide (NO). Leukocyte infiltration is the most important factor in the development of obstructive nephropathy. Also free oxygen radicals have been reported to play an important role in the development of renal damage(7).

Sugammadex is a newly developed agent that is used for the rapid reversal the effects of certain non depolarizan muscle remaxant agents (NDMR) such as rocuronium and vecuronium which are steroid structured agents. Two different doses of sugammadex (2 $mg.kg^{-1}$ and 4 $mg.kg^{-1}$) have been shown as safe doses for human(8).Hypotension, coughing and anaphylaxis are reported side effects of sugammadex(9). Also elongated clotting time with sugammadex have been reported. However contraray results have been reported related with effects of sugammadex on clotting time(10).

Sugammadex is excreeted by the kidneys and so its use is not recommended in renal failure. Chronic renal failure (CRF) is considered as a major health problem all around the world and incidence of CRF is gradually increasing. It is estimated that 13% of adults in the United States suffer from this disease. Similarly a study from Beijing shows that morbidity rate is 13% in China(3-5).

Previous study we investigated the effect of high-dose sugammadex on erythrocyte deformability of UUO in rats(11).

In this study we aimed to investigate the effect of sugammadex on kidney tissue -which is a cyclodextrin developed to recover the block created by rocuronium and vecuronium- on the rats with experimentally created early or late stage of kidney failure using experimental UUO model.

## MATERIALS and METHODS

*Animals and Experimental Protocol*

After obtaining approval of the Experimental Animals Ethics Committee of Gazi University, we conducted the study in the GUDAM Laboratory of Gazi University. All the experimental procedures were performed in conjunction with Guide for the Care and Use of Laboratory Animals standards.

The animals were housed in a pathogen-free environment, with free access to food (until 2 h before the anaesthetic procedure) and water and with a 12 h light/dark cycle for at least one week before study. Thirty male Wistar albino rats (225 and 300 gr) were randomly divided into five groups. Control (C) Group (control; n=6), Unilateral Ureteral Obstruction (UUO) for 1 week Group (UUO-1; n=6), UUO-1 week treated with rocuronium and 96 $mg.kg^{-1}$ Sugammadex Group (UUO-1 week-96S, UUO-1-rocuronium-96 $mg.kg^{-1}$ sugammadex; n=6), UUO for 3 weeks Group (UUO-3; n=6) and UUO-3 weeks treated with rocuronium and 96 $mg.kg^{-1}$ Sugammadex Group UUO-3 week-96S (UUO-3- rocuronium-96 $mg.kg^{-1}$ sugammadex; n=6). Rats in the control and UUO groups received only normal saline at the same volume.

Renal failure was induced in the left ureter in four groups of rats under ketamine anesthesia with a low abdominal incision, and then the suture was placed with 2.0 mersilene and was kept there for 1 week for the early period, and 3 weeks for the late period.

At the end of 1 week for the early period and 3 weeks for the late period, anesthesia was induced using 100 $mg.kg^{-1}$ ketamine intraperitoneally and tracheostomy was performed in all the animals via 12G cannula; intubation was performed. Ventilation was maintained by a ventilatory machine at room temperature. The ventilatory machine was adjusted at a rate 70-100/min, tidal volume 2.5-3 ml and positive end-expiratory pressure 2 mmHg.

After the reversal of the muscle-relaxant effects of rocuronium using sugammadex, rats were allowed to breathe spontaneously. When needed anaesthesia was maintained by repetitive injections of 20 $mg.kg^{-1}$ ketamine.

After the 30 minutes of follow-up period, 100 $mg.kg^{-1}$ ketamine was administered intraperitoneally then rats in study groups (UOO-1 week, UOO-1week 96S, UOO-3 weeks, UOO-3 weeks 96S) were sacrificed after collecting blood samples from their abdominal aorta. The blood samples are stored at -20 ° C subsequently MDA and NO were measured in serum. Kidney tissues on both kidneys were taken and stored in 10% formalin for histopathological examination.

Measurements of MDA levels and NO activities

Lipid peroxidation was measured using Esterbauer method. Briefly Malondialdehyde reacted with thyobarbutiric acid at 90-95 C degree and resulted in pink chromogranin. Following this procedure specimens rapidly cooled then absorbances were read at 532 nm spectrophotometrically. Results were represented as nmol/g tissue protein.[12] Stable oxidative NO metabolites ($NO_2$- ve $NO_3$ -) were measured in serum also NO production was measured. Griess reaction was used in order to measure nitrite concentration.[13]

*Histopathological examination*

Histopathological examination was held at Kirikkale University, Histology and Embryology Department of Faculty of Medicine. After routine fixing process of the kidneys, 5 µ 'hood sections performed in paraffin blocks, they were stained by Hematoxylin & Eosin (H&E) and then examined by light microscope. For histopathological evaluation, the scoring table of Bostan et al. was used(14). Each kidney preparation were evaluated in a similar manner in terms of damage criteria for glomerular vacuolization (GV), tubular dilatation (TD), Vascular vacuolisation and hypertrophy (VVH), tubular cell degeneration and necrosis (TCDN), Bowman's space dilatation (BSD), tubular hyaline cylinders (THC), lymphocyte infiltration (LI), tubular cell sloughing (TCS). 4-point scoring system was used. They were rated as 0: No change. +1: Minimal change. +2: Moderate change. +3: Serious change.

*Statistical Analysis*

A package programme of SPSS 20.0 was used in order to perform statistical analysis. The findings were expressed as mean ± standard error (mean±SE). The data were evaluated using Kruskal-Wallis variance analysis. The variables with significance were evaluated using a Bonferroni corrected Mann-Withney U test. $p<0.05$ was considered statistically significant.

## RESULTS

Kidney glomerular vacuolization (GV) levels, on the side (left) that we don't applied obstruction, were found significantly different between groups on light microscopy (p=0.026). GV level in UUO3 and UUO3S was found more than the control group (p=0.035 and p=0.004, respectively) (Table 1, Figure 1-5). Additionally, the GV in UUO3S group was determined more than UUO1 group (p=0.012).

Tubular dilatation (TD) between left kidney tissues were found significantly different among the groups (p=0.046). In UUO1, UUO3, and UUO3S groups TD was observed more than the control group (p=0.040 and p=0.012, p=0.011, respectively) (Table 1, Figure 1-5).

**Table 1.** Left kidney (normal) tissue histopathology [Mean ± SE]

| | Group C (n=6) | Group UUO1 (n=6) | Group UUO1S (n=6) | Group UUO3 (n=6) | Group UUO3S (n=6) | P** |
|---|---|---|---|---|---|---|
| **Glomerular vacuolization (GV)** | 0.0±0.0 | 0.17±0.17 | 0.33±0.33 | 0.60±0.25* | 0.83±0.17*,& | 0.026 |
| **Tubular dilatation (TD)** | 0.50±0.22 | 0.83±0.17 | 1.00±0.00* | 1.17±0.17* | 1.33±0.33* | 0.046 |
| **Vascular vacuolisation and hypertrophy (VVH)** | 0.33±0.21 | 0.83±0.31 | 1.33±0.66 | 0.33±0.21 | 0.50±0.34 | 0.285 |
| **Tubular cell degeneration and necrosis (TCDN)** | 0.0±0.0 | 0.33±0.21 | 0.67±0.33* | 0.50±0.22 | 0.83±0.17* | 0.042 |
| **Bowman's space dilatation (BSD)** | 0.40±0.25 | 0.33±0.21 | 0.33±0.33 | 0.20±0.20 | 0.83±0.17 | 0.279 |
| **Tubular hyaline cylinders (THC)** | 0.33±0.21 | 0.67±0.21 | 1.00±0.00 | 0.40±0.24 | 0.83±0.17 | 0.205 |
| **Lymphocyte infiltration (LI)** | 0.67±0.21 | 0.83±0.17 | 1.00±0.00 | 0.67±0.21 | 1.17±0.17 | 0.279 |
| **Tubular cell** sloughing **(TCS)** | 0.67±0.21 | 1.00±0.00 | 1.00±0.00 | 1.00±0.00 | 0.83±0.17 | 0.312 |

p**: Statistical significance was set at a p value < 0.05 for Kruskal-Wallis test
* *p*<0.05 compared to Group C; &*p*< 0.05 compared to Group UO1

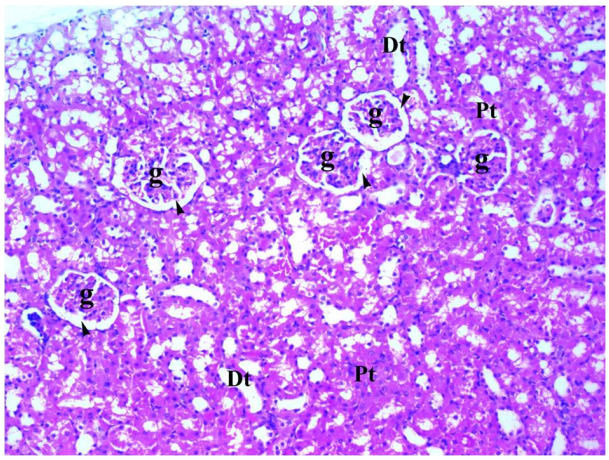


**Figure 1:** Left renal tissue Control group (g: glomerul; arrowhead: bowman's gap; Dt: distal tubule; Pt: proximal tubule)

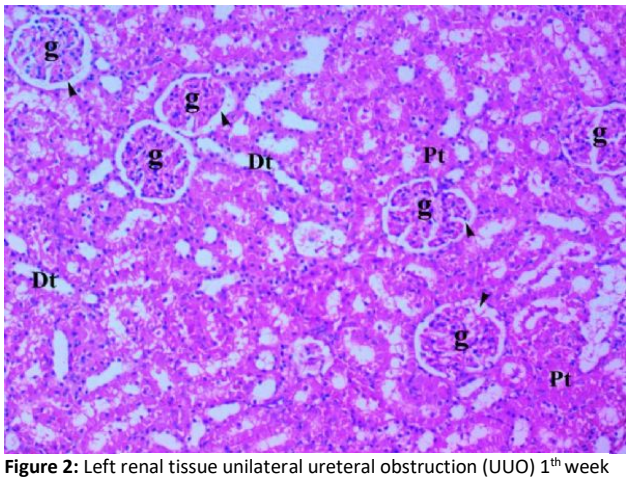


**Figure 2:** Left renal tissue unilateral ureteral obstruction (UUO) 1th week (g: glomerul; arrowhead: bowman's gap; Dt: distal tubule; Pt: proximal tubule)

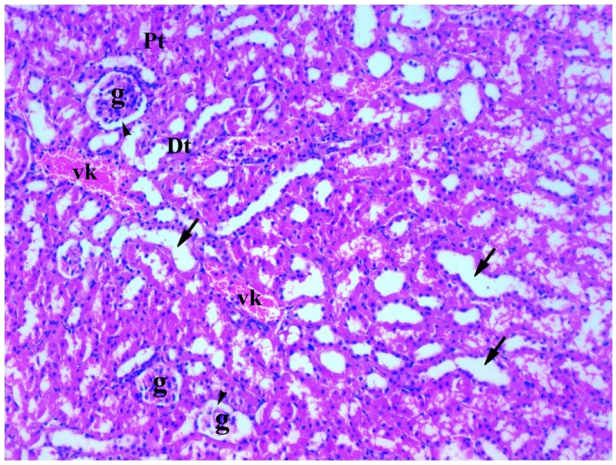


**Figure 3:** Left renal tissue UUO1 Sugammadex (g: glomerul; arrowhead: bowman's gap; arrow: dilatation tubuls; Dt: distal tubule; Pt: proximal tubule; vc: vascular congestion)

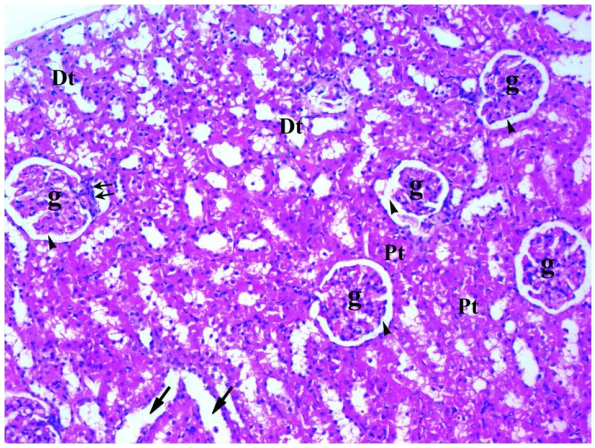


**Figure 4:** Left renal tissue UUO3 (g: glomerul; arrowhead: bowman's; arrow: dilatation tubuls; Dt: distal tubule; Pt: proximal tubule; double arrow: macula densa)

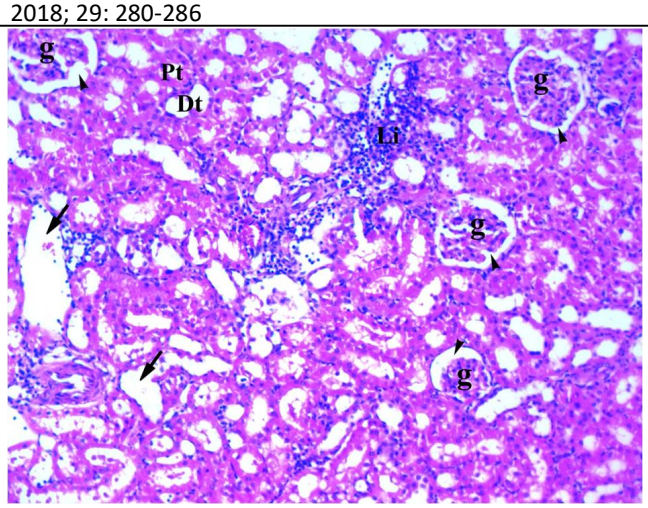


**Figure 5:** Left renal tissue UUO3 Sugammadex (g: glomerül; arrowhead: bowman's; arrow: dilatation tubuls; Dt: distal tubule; Pt: proximal tubule; Li: lymphcocyte infiltration)

Tubular cell degeneration and necrosis (TCDN) were also found significantly different in the left kidney tissue among the groups (p=0.042). TCDN was found more in the UUO1S and UUO3S groups in comparison with control group (p=0.045 and p=0.004, respectively), (Table 1, Fig 1-5).

When we examine the tissue that the right kidney obstruction was applied; glomerular vacuolization (GV) levels were found significantly different between groups (p=0.003). GV were significantly found more in UUO1S, UO3 and UUO3S groups than the control group (p=0.020, p=0.003, p<0.0001, respectively) (Table 2, Fig 6-10).

Tubular dilatation (TD) between the right kidney tisues were also found significantly different (p<0.0001). TD was observed in all groups higher than the control group (p<0.0001, all) (Table 2, Figure 6-10).

Vascular vacuolization and hypertrophy (VVH) was found significantly different between groups (p<0.0001) in the obstruction applied side of the kidney tissues. In all study groups VVH scores were significantly higher than that in the control group (p<0.0001, all), (Table 2, Fig 6-10). Additionally, VVH in the UUO3S group was found more than the UUO1 and UUO1S groups (p=0.008) (Table 2).

**Table 2.** Right kidney (ureteral obstruction was created) tissue histopathology [Mean ± SE]

| | Group C (n=6) | Group UUO1 (n=6) | Group UUO1S (n=6) | Group UUO3 (n=6) | Group UUO3S (n=6) | P** |
|---|---|---|---|---|---|---|
| **Glomerular vacuolization (GV)** | 0.0±0.0 | 0.67±0.33 | 1.00±0.58* | 1.33±0.33* | 1.50±0.22* | 0.003 |
| **Tubular dilation (TD)** | 0.50±0.22 | 2.33±0.33* | 2.67±0.21* | 3.00±0.00* | 3.00±0.00* | <0.0001 |
| **Vascular vacuolisation and hypertrophy (VVH)** | 0.33±0.21 | 2.00±0.00* | 2.00±0.00* | 2.67±0.21* | 3.00±0.00*,&,? | <0.0001 |
| **Tubular cell degeneration and necrosis (TCDN)** | 0.00±0.00 | 1.00±0.00* | 2.00±0.36*,& | 2.00±0.00*,& | 2.00±0.00*,& | <0.0001 |
| **Bowman's space dilatation (BSD)** | 0.00±0.00 | 0.50±0.22 | 0.67±0.33 | 1.17±0.31* | 1.33±0.33* | 0.003 |
| **Tubular hyaline cylinders (THC)** | 0.00±0.00 | 1.00±0.00* | 1.67±0.33* | 1.00±0.00* | 1.67±0.33* | <0.0001 |
| **Lymphocyte infiltration (LI)** | 0.67±0.21 | 2.00±0.00* | 2.33±0.33* | 2.67±0.21* | 2.67±0.33* | <0.0001 |
| **Tubular cell sloughing (TCS)** | 0.50±0.22 | 1.00±0.00 | 1.67±0.33* | 2.00±0.00*;& | 3.00±0.00*,& | <0.0001 |

p**: Statistical significance was set at a p value < 0.05 for Kruskal-Wallis test
* *p*<0.05 compared to Group C; &*p*< 0.05 compared to Group UO1; ?p<0.05: compared to Group UO1S

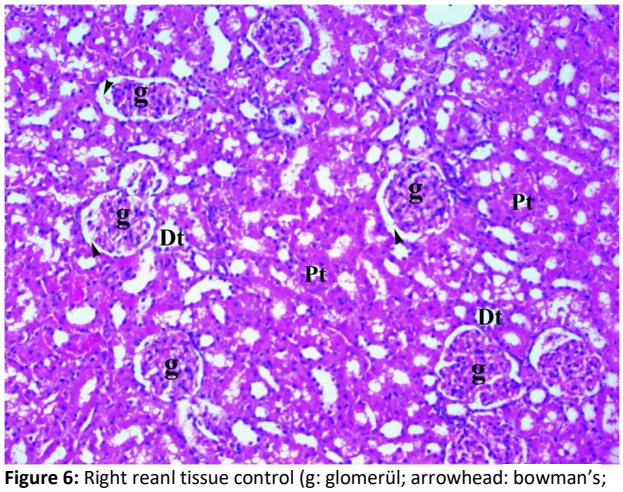


**Figure 6:** Right reanl tissue control (g: glomerül; arrowhead: bowman's; Dt: distal tubule; Pt: proximal tubule)

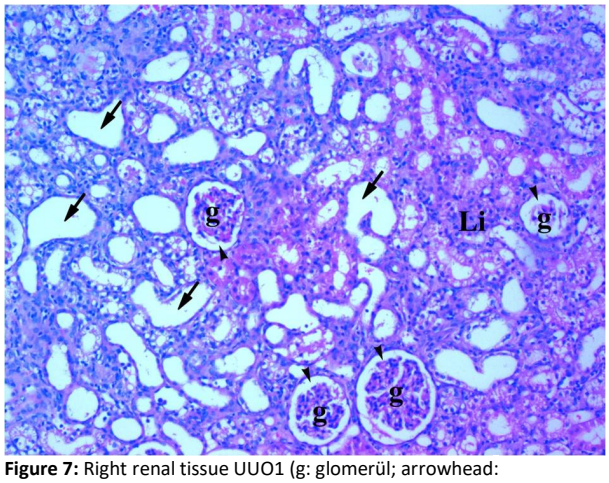


**Figure 7:** Right renal tissue UUO1 (g: glomerül; arrowhead: bowman's; arrow: dilatation tubuls; Li: lymphcocyte infiltration)

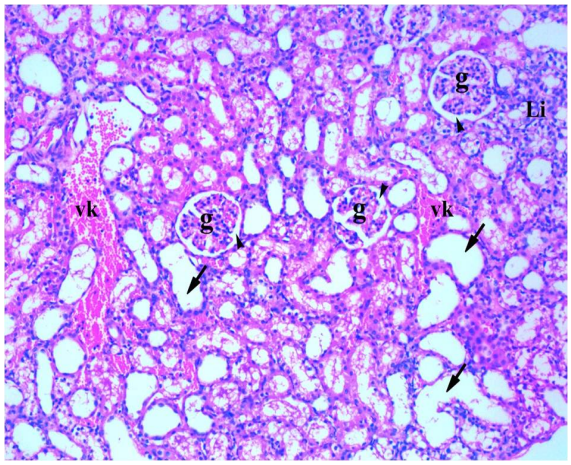


**Figure 8:** Right renal tissue UUO1 Sugammadex (g: glomerül; arrowhead: bowman's; arrow: dilatation tubuls; Li: lymphcocyte infiltration vc: vascular congestion)

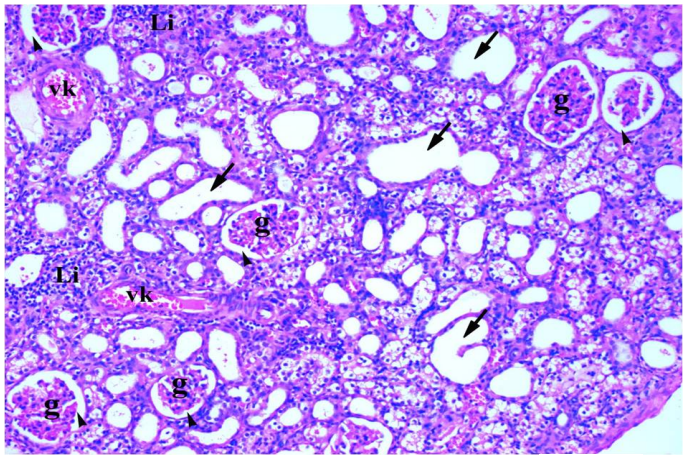


**Figure 9:** Right renal tissue UUO3 (g: glomerül; arrowhead: bowman's; arrow: dilatation tubuls; Li: lymphcocyte infiltration vc: vascular congestion)

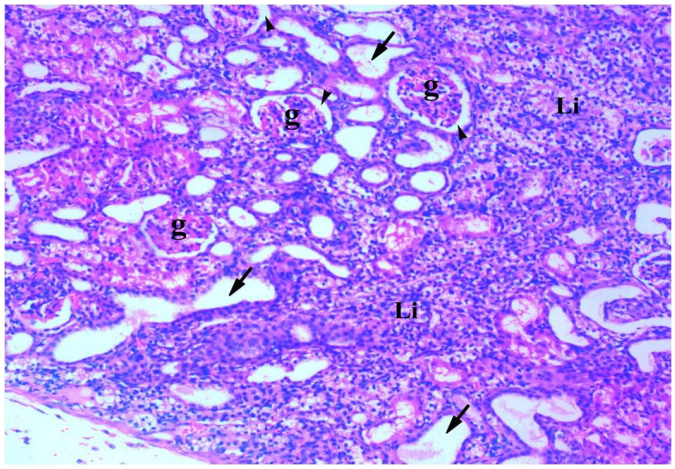


**Figure 10:** Right renal tissue UUO3 Sugammadex (g: glomerül; arrowhead: bowman's; arrow: dilatation tubuls; Li: lymphcocyte infiltration vc: vascular congestion)

Tubular cell degeneration and necrosis (TCDN) was found significantly different among the groups similarly as we examine the right kidney tissues (p<0.0001). It was observed in all groups more TCDN than the control group (p<0.0001, all), (Table 2, Fig 6-10).

Also TCDN in UUO1S, UUO3 and UUO3S groups were observed more than the UUO1 group (p=0.026 and p=0.026, p=0.02, respectively) (Table 2, Figure 6-10).

When the right kidney tissue examined, bowman space dilatation (BSD) was observed significantly different between the groups (p=0.003). BSD in the UUO3 and UUO3S groups was observed more than the control group (p=0.002, p=0.001, respectively) (Table 2, Figure 6-10).

Tubular hyaline cylinders (THC) were found significantly different among the groups in the side that the obstruction was applied (p<0.0001). THC was observed in all groups more than the control group (p=0.012, p<0.0001, p=0.012, p<0.0001, respectively) (Table 2, Figure 6-10).

Lymphocyte infiltration (LI) were found significantly different among the groups that right kidney tissue was studied (p<0.0001). In all groups LI was observed more than the control group (p=0.002, p<0.0001, p<0.0001, p<0.0001, respectively) (Table 2, Figure 6-10).

Tubular cell sloughing (TCS) were also observed significantly different among the groups which the obstruction was applied (p<0.0001). TCS in the UUO1S, UUO3 and UUO3S groups was observed more than the control group (p<0.0001, all), (Table 2, Figure 6-10). Also, TCS in the UUO3 and UUO3S groups was observed more than UUO1 group (p<0.0001, all), (Table 2, Figure 6-10).

Serum MDA enzyme activity was significantly different between the groups (p=0.026). MDA enzyme activities of the UUO1S, UUO3 and UUO3S groups were found to be significantly higher than the control group (p=0.021, p=0.025, p=0.002, respectively) (Table 3).

When the groups were compared in terms of serum NO enzyme activity, significant difference was found between the groups (p=0.004). NO enzyme activity of UUO1S, UUO3 and UUO3S groups was found to be significantly lower than the control group (p=0.005 and p=0.011, p<0.0001, respectively) (Table 3).

**Table 3.** Oxidant status parameters [Mean ± SE]

| | Group C (n=6) | Group UUO1 (n=6) | Group UUO1S (n=6) | Group UUO3 (n=6) | Group UUO3S (n=6) | P** |
|---|---|---|---|---|---|---|
| **MDA (nmol/mg prot)** | 8,44±1,43 | 19,22±9,35 | 22,91±7,68* | 22,42±8,96* | 29,03±17,02* | 0.026 |
| **NO (IU/mg prot)** | 15,11±4,58 | 11,69±3,43 | 8,98±3,02* | 9,57±3,60* | 6,53±2,64* | 0.004 |

p**: Statistical significance was set at a p value < 0.05 for Kruskal-Wallis test
* *p*<0.05 compared to Group C

**DISCUSSION**

In this study, effects of high doses sugammadex on kidney tissue of the rats those have early and late phase renal failure induced by UUO was investigated.

Sugammadex is a kind of new generation cyclodextrin derivative agent that is used for reversing steroid structure neuromuscular blocking agents (NMBA). It forms inclusion complexes by taking the lipophilic NMBAs inside (molecular encapsulation) with high affinity. This prevents steroidal NMBA binding to receptors. This water soluble complex transits to plasma by way of diffusion and NMBA concentration in free blood concentration decreases(15-17). Sugammadex is cleared by way of kidney. Despite the slowdown of the excretion of sugamadeks in end-stage renal failure, it was found quick and effective for reversing the rocuronium block(18).

In a study published by the Lobaz and friends, sugammadex has reversed the block quickly in a patient with advanced-stage renal disease(19). In another study, the sugammadex-rocuronium complex discharge was found to be prolonged in advanced-stage renal disease(20).

Staals et al 's study has also shown that although it is well tolerated, 2 $mg.kg^{-1}$ use of sugammadex at the patients with severe renal failure compared to healthy patients, plasma clearance has reduced(18,20). It was found in another study that sugammadex at 4 $mg.kg^{-1}$ dose in 35 patients with severe renal failure for 4 weeks was well tolerated. However; sugammadex complex has been determined at blood samples up to 7 days in patients of renal failure(21). Unlikely, Yokota et al have reported in their study that rocuronium-sugammadex complex was excreted with the urine within 24 hours(22).

In a study, which 30 patients were evaluated and patients with renal failure were compared with the patients with normal renal function, 2 $mg.kg^{-1}$ sugammadex was used and monitoring was made by train of four (TOF). After monitorization, although in advanced kidney disease patients it was determined to be reversed more slowly, it was not accepted as statistically meaningfull. It was stated that this difference was not related with the renal excretion but it was due to the time difference between the bindings of the complex(23).

We brought our work by creating unilateral ureter obstruction and causing kidney damage. 96 $mg.kg^{-1}$ sugammadex was applied for early and late periods (1-week for early period and 3-weeks for late period) and we evaluated the histological changes that occured in rats.

Bostan et al. divided 36 rats into 6 groups; they investigated the effects of only sugammadex of 16 $mg.kg^{-1}$ and 96 $mg.kg^{-1}$ doses; sugammadex-rocuronium and only rocuronium on the rat kidney histopathology and biochemical parameters by comparing with the control group. 72 hours after the administration of medicins, endothelial and mesenchymal cells were identified in normal kidney tissue. As a result of cell degeneration and necrosis, they observed cytoplasmic vacuolization, cell death and Bowman capsule dilatation. Proximal and distal tubules in all groups except the group which was given 96 $mg.kg^{-1}$ of sugammadex identified as normal. In the groups which sugammadex and rocuronium administered together, the inflammation was found to be more, especially lymphocytes and eosinophils. No significant difference was obsereved in the formation of tubular hyaline cylinders. No significant changes was observed in biochemical markers (urea, creatinine and electrolytes) in high doses of sugammadex, and for 96 $mg.kg^{-1}$ of sugammadex it was observed that early spontaneous respiration began and both doses of sugammadex were reported to be effective(14).

Similar to the study of Bostan et al., we evaluated the parameters of glomerular vacuolization (GV), tubular dilatation (TD), vascular vacuolisation and hypertrophy (VVH), tubular cell degeneration and necrosis (TCDN), bowman space dilatation (BSD), tubular hyaline cylinders (THC), lymphocyte infiltration (LI), tubular cell sloughing (TCS). We used 4 point scoring system. A score of 0-3 was given. 0: no change, +1: uncertain change, +2: slight change, +3: was graded as severe change. Significant difference was found in all cases at the kidney on the side of the obstruction. For the kidney tissues which obstruction was not applied, GV in all the groups with 3 weeks was determined significantly more than the control group. TD in UUO1, UUO3 and UUO3S groups was observed higher than that in the control group. And TCDN in UUO1S and UUO3S groups was observed higher than that in the control group.

Grande et al. examined kidneys of the rats with experimental renal failure and showed that mild fibrosis was observed from end of the first week, interstitial fibrosis became apparent at the end of the second week and a mild dysfunction of the kidneys appeared at the end of two weeks(24).

Dursun et al. observed a significant increase on serum urea and creatinine levels in the rats with urether obstruction created experimentally and showed amorphous eruptions, cellular swelling, vacuolization, neutrophile in the glomerules, tubular dilatation and increase in vascular congestion parameters in the tubular lumen histopathologically(25).

In the present study, a significant difference was found between the groups in terms of all histopathological parameters analyzed in the kidney where urether obstruction was created. GV and TD were observed more in groups UUO1S, UUO3 and UUO3S when compared with the control group. TD, VVH, TCDN, LI and THS were observed more in all groups than the control group. BSD was detected more in groups UUO3and UUO3S when compared with the control group. Consequently, we believe that we provided a sufficient ureter obstruction and renal injury.

Other studies performed on histopathology of the kidney also showed that sugammadex is primarily eliminated by the kidneys(20,26). Szenohradszky et al. clearly stated that the effect was dependent to the dose.[26] An increased exposure to drug delivery complex may cause histopathological degradation in the kidneys. Similarly, Bostan et al. concluded that increased sugammadex dose caused such histopathological modification. However, they observed more significant histopathological modifications on the rats that they administrated rocuronium only when compared with the rats that they administrated sugammadex; and considered that slight effects of sugammadex on the kidneys might increase the degenerative effect of rocuronium. Furthermore, they did not detect any statistically significant difference on serum urea, creatinine, sodium and potassium concentrations(14).

We believe that sugammadex administration caused histopathological modification which is more significant on late term on the rats that we have created kidney failure. Our findings support the studies conducted by Bostan et al. who administrated high dose of sugammadex. We consider that histopathologhical injury effect of sugammadex increase by prolongation of kidney failure.

Devarajan et al. concluded in their study that sugammadex and sugammadex-rocuronium complex are excreted by the urine without binding to plasma proteins and erythrocytes(23).

One of the most important mechanisms in free radical-induced cell injury is lipid peroxidation(27). Although MDA appeared as a result of oxidation of polyunsaturated fat acids on the membrane is not specific, it is one of the most important indicators for lipid peroxidation through a well correlation with the grade(28).

Chronic inflammation is a common condition for the patients with CKD, especially those on dialysis therapy (HD, peritoneum dialysis-PD)(29). It is known that SOR produced excessively by active neutrophilles causes tissue injyry in inflammatory diseases(30). MDA which degraded aminophospholipid organization of the erythrocyte membrane was shown to be effective on cell injury(31).

A study performed unilateral ureter obstruction in half of 80 rats included into the study, removed the renal tissues and examined at the end of weeks 2 and 4 and detected that the oxidative stress progressively increased as well as antioxidants decreased as a result of ureter obstruction for 4 weeks. MDA values were detected significantly higher in terms of lipid peroxidation, an indicator for oxidative stress(32). In line with the previous studies, renal fibrosis and apoptosis caused by oxidative stress due to ureter obstruction were observed(32-34).

Similarly, Grande et al. measured MDA which is accepted as an indicator for SOR activity on the rats that created renal failure and showed a significant increase(24).

Similar to other studies, when the groups were compared each other, we found MDA enzyme activity higher in groups UUO1S, UUO3 and UUO3S than the control group. We believe that sugammadex administration caused an increase in MDA enzyme activity which is more significant on late term on the rats that we have created kidney failure. We consider that MDA enzyme activity increasing effect of sugammadex increase by prolongation of kidney failure. Nitric Oxide (NO) is a lipophilic free radical appeared by NOS enzyme activity. NO reacts with the superoxide in case of increase of superoxide and creates peroxynitrite which is a pro-oxidant whereas acts like an antioxidant in some cases and protects the cell from lipid peroxidation(35). NOS level is an indicator which may be used to evaluate the effect of the oxidative stress on endothelial functions(36). NO protects the cell from reperfusion injury of ischemia through vasodilatation(37).

Aiello et al. reported that increased iNOS and eNOS values supported increased NO release from vascular wall in uremic patients(38). In-vivo NO levels increase in HD patients. Although NO is a cytotoxic molecule responsible from dialysis complications, NO and creatinine are the parameters that may be followed prognostically in the patients with chronic kidney diseases who are on dialysis(39).

Differently, Kılıc et al. reported that NO effect varied and such effect appeared due to useful hemodynamic effects of NO as well as the balance between the cytotoxicity appeared in the kidney and other tissues(40).

In the present study, NO enzyme activity was found significantly lower in groups UUO1S, UUO3 and UUO3S than the control group. In the present experimental study where UUO and early and late term kidney failure were created, histopathological comparison of high sugammadex dose effect on renal tissue revealed the following outcomes;

1. We believe that sugammadex administration caused histopathological modification which is more significant on late term on the rats that we have created kidney failure. Our findings support the studies conducted by Bostan et al. who administrated high dose of sugammadex. We consider that histopathologhical injury effect of sugammadex increase by prolongation of kidney failure.
2. We believe that sugammadex administration caused an increase in MDA enzyme activity which is more significant on late term on the rats that we have created kidney failure. We consider that MDA enzyme activity increasing effect of sugammadex increase by prolongation of kidney failure.
3. High dose sugammadex administration caused a significant histopathological injury on the kidney that ureter obstruction was created, as more significant during early and late term. However, it was detected that high dose sugammadex administration caused a mild histopathological injury on the kidney without ureter obstruction. We consider that it may be used safely in case of uniletaral ureter obstruction; however use in bilateral ureter obstruction should be watched out.
4. We predict that MDA and NO activity change as more significant in advanced stage kidney failure and high dose sugammadex administration may contribute to the oxidative stress.

We consider that it may be used safely in case of UUO; however use in bilateral ureter obstruction should be watched out.

**Conflict of interest**

No conflict of interest was declared by the authors.

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