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ORIGINAL RESEARCH

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An animal model of reperfusion in ischemic corporal tissue and the effect of sugammadex on oxidative injury parameters

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Abstract

Background: The aim of this study was to analyze the effect of sugammadex on ischemia-reperfusion injury in corporal tissue. **Methods**: Eighteen male Wistar Albino strain rats were divided into three groups. Group 1 served as the control group. A priapism model was induced in rats in Group 2 and 3. Rats in Group 3 were additionally administered 4 mg/kg sugammadex intravenously immediately after reperfusion. All rats underwent penectomy for histopathological and biochemical evaluations, and blood samples were collected. **Results**: In Group 2, total oxidant status (TOS) and malondialdehyde (MDA) levels were significantly higher compared to other groups ($p < 0.001$ and $p = 0.009$, respectively). A substantial reduction in both TOS and MDA levels was found in Group 3 ($p < 0.001$ and $p = 0.043$, respectively). Group 2 exhibited significantly lower activities of a glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) compared to Group 1 ($p = 0.043$ and $p = 0.024$, respectively). In contrast, Group 3 showed significant increases in both antioxidant enzyme activities ($p = 0.036$ and $p = 0.034$, respectively). Similarly, the total antioxidant status (TAS) was dramatically higher in Group 3 compared to Group 2 ($p < 0.001$). Microscopic examinations revealed improvements in vasocongestion, edema, desquamation and inflammation scores in Group 3 compared to Group 2 ($p < 0.001$). **Conclusions**: Biochemical and histopathological findings suggest that, sugammadex significantly mitigates oxidative damage parameters in corporal tissue affected by ischemia-reperfusion injury.

Keywords

Sugammadex; Ischaemia-reperfusion injury; Corporal tissue; Rat

Un modelo animal de reperfusión en tejido corporal isquémico y el efecto del sugammadex sobre los parámetros de lesión oxidativa

Resumen

Antecedentes: El objetivo de este estudio fue analizar el efecto del sugammadex sobre la lesión por isquemia-reperfusión en el tejido corporal. **Métodos**: Dieciocho ratas macho de la cepa Wistar Albino se dividieron en tres grupos. El grupo 1 sirvió como grupo control. Se indujo un modelo de priapismo en las ratas de los grupos 2 y 3. A las ratas del Grupo 3 se les administró adicionalmente 4 mg/kg de sugammadex por vía intravenosa inmediatamente después de la reperfusión. Todas las ratas se sometieron a penectomía para evaluaciones histopatológicas y bioquímicas, y se recolectaron muestras de sangre. **Resultados**: En el Grupo 2, el estado oxidante total (TOS) y los niveles de malondialdehído (MDA) fueron significativamente más altos en comparación con otros grupos ($p < 0.001$ y $p = 0.009$, respectivamente). Se encontró una reducción sustancial en los niveles de TOS y MDA en el Grupo 3 ($p < 0.001$ y $p = 0.043$, respectivamente). El Grupo 2 exhibió actividades significativamente menores de una glutatión peroxidasa (GSH-Px) y superóxido dismutasa (SOD) en comparación con el Grupo 1 ($p = 0.043$ y $p = 0.024$, respectivamente). Por el contrario, el Grupo 3 mostró aumentos significativos en ambas actividades enzimáticas antioxidantes ($p = 0.036$ y $p = 0.034$, respectivamente). De manera similar, el estado antioxidante total (TAS) fue dramáticamente más alto en el Grupo 3 en comparación con el Grupo 2 ($p < 0.001$). Los exámenes microscópicos revelaron mejoras en las puntuaciones de vasocongestión, descamación, edema e inflamación en el Grupo 3 en comparación con el Grupo 2 ($p < 0.001$). **Conclusiones**: Los hallazgos bioquímicos e histopatológicos sugieren que el sugammadex mitiga significativamente los parámetros de daño oxidativo en el tejido corporal afectado por lesión por isquemia-reperfusión.

Palabras Clave

Sugammadex; Lesión por isquemia-reperfusión; Tejido corporal; Rata

1. Introduction

Priapism is defined as a penile erection lasting longer than four hours and occurring independently of sexual stimulation [1]. There are various types of priapism, each with its unique pathophysiology and management approach. Ischemic priapism is referred to as a compartment syndrome affecting the penis and is considered a true urological emergency. It is characterized by a decrease in venous outflow and vascular stasis, which leads to tissue hypoxia. Management of ischemic priapism should be addressed as a matter of urgency [1, 2]. Ischemic priapism lasting longer than 24 hours is associated with erectile dysfunction in 90% of cases [2]. The management of this condition focuses on three main goals: resolving the acute period, restoring oxygenation to penile tissue, preserving sexual function and preventing relapse [1, 2].

However, the initiation of treatment algorithms to restore detumescence often leads to a sudden and excessive increase in oxygen within the corpora cavernosa, resulting in the generation of reactive oxygen species (ROS). ROS trigger a series of reactions known as ischemia-reperfusion injury, which causes significant harm to vital cellular components. It is crucial to protect erectile tissues from the damaging consequences of ischemia-reperfusion injury in order to preserve sexual health [1].

Sugammadex is widely used in operating rooms to reverse the effects of steroidal muscle relaxants, such as vecuronium and rocuronium [3]. This agent, which has a modified gamma-cyclodextrin structure, has been documented in past experimental studies to exhibit anti-inflammatory and antioxidant effects, thereby preventing oxidative stress-induced damage [4]. In these contexts, sugammadex has proven effective in protecting various tissues, such as the stomach, extremities, ovaries, brain and kidneys, from ischemia-reperfusion injury [4–8].

This experimental study, the first of its kind in the English literature, aimed to assess the potential protective role of sugammadex in preventing corporal ischemia-reperfusion injury in a rat model of priapism.

2. Material and method

2.1 Experimental method

After obtaining approval from the local ethics committee for animal experiments (Tokat Gaziosmanpaşa University Animal Studies Ethics Committee Decision No: 2024-HADYEK-12, Date: 07 August 2024), eighteen male Wistar rats, weighing 220–400 g and aged 2–3 months were used in this study. The animals were housed under standard vivarium conditions in a climate-controlled room (12-h light/dark cycle and 18–22 °C), with free access to water and standard rodent chow. All surgical intervention were carried out in a sterile environment with appropriate anesthesia. Anesthesia was induced using intraperitoneal ketamine at a dose of 50 mg/kg.

Experimental animals were divided into three groups.

Group 1 (Control Group): This group underwent only a penectomy, and blood samples were obtained from the inferior vena cava.

Group 2 (Ischaemia-Reperfusion Group): This group underwent ischemia-reperfusion injury without treatment.

Group 3 (Treatment Group): This group received sugammadex treatment after reperfusion.

In Groups 2 and 3, a priapism model was established as previously described in the literature. For this procedure, Foley catheters were cut into uniform lengths of approximately 2 mm to form constriction bands. Penile erection was achieved in rats by applying negative pressure to the penis using a 50 cc

syringe. Constriction bands were then positioned at the root of the penis, and the priapism model was established. The constriction bands were kept in place for one hour before being removed. To assess ischemia-reperfusion injury, penectomy was performed, and blood samples were drawn from the inferior vena cava after one hour of reperfusion [9]. In this context, penile tissues were exposed to ischemia-reperfusion injury for one hour. Half of the penile tissues were used for biochemical analysis, while the other half was used for histopathological examination. Additionally, blood biochemical analysis was performed on the experimental animals.

In Group 3, unlike the other groups, 4 mg/kg sugammadex was administered intravenously immediately after reperfusion [5].

2.2 Biochemical analysis

2.2.1 Preparation of samples

Penile tissue was homogenized with a pH 7.4 Tris-hydrogen chloride (HCI) phosphate buffer solution and a homogenizer to assess the activity of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) enzymes, along with malondialdehyde (MDA) levels. Part of the homogenate was reserved to prepare the supernatant, while the remaining portion was retained as a homogenate. MDA levels were examined in the homogenate, while SOD, GSH-Px activities were measured in the supernatant, which was obtained by centrifuging the homogenate at 4 °C for 15 minutes at 4000 rpm [10–14]. For blood biochemical analysis, blood collected from the inferior vena cava of the rats was placed in tubes and centrifuged at +4 °C for 5 minutes at 4000 rpm to obtain serum. The serum was then used to measure total oxidant status (TOS) and total antioxidant status (TAS) levels [15, 16].

2.2.2 Measurement of SOD, GSH-Px and MDA

MDA, a marker of lipid peroxidation, was measured using the method described by Esterbauer and Cheeseman [12].

SOD activity, which reflects its role in protecting against oxidative stress, was evaluated according to the method described by Sun *et al*. [13].

GSH-Px activity was assessed using the method outlined by Paglia *et al*. [14].

In the results, SOD and GSH-px activities units were expressed as U/g protein, while MDA levels were expressed as μmoL/g wet tissue.

2.2.3 Measurement of TOS and TAS

TAS and TOS levels were determined using a spectrophoto metric kit (KM24156A/KM241730, Rel Assay Diagnostics, Gaziantep, Turkey) [15, 16]. Measurements of TAS and TOS were performed on an autoanalyzer (Beckman Coulter LX 20, Inc., Fullerton, CA, USA). Serum levels were reported as millimolar Trolox equivalent per liter (L) [17].

2.3 Histopathological evaluation

Penile tissues from the experimental animals were fixed in 4% buffered neutral formalin (TK.030408.05001, Tekkim KimyaTekkim Kimya, Bursa, Turkey) for 48 hours and then embedded in paraffin blocks. Thin sections (5 μm thick) of the paraffin-embedded penile tissues were cut using a rotary microtome (RM2135, Leica, Nussloch, Germany). The thin serial sections were mounted on slides and stained according to the hematoxylin-eosin staining protocol to prepare them for microscopic examination. The stained penile tissue slides were evaluated using a research light microscope (Nikon Eclipse 200, Nikon, Yokohama, Japan) at 40× magnification and scored in the NIS-Element program (Nikon 471, Nikon, Yokohama, Japan) by a single expert histopathologist, blinded to the experimental groups. Histopathological analyses were performed by evaluating images from 5 different areas, with an average of 5–6 consecutive sections from the penile tissues of each individual. Tissue slides were evaluated and scored based on desquamation, vasocongestion, edema and inflammation, using the following scoring system 0 = normal, 1 = mild, 2 = moderate and 3 = severe [18].

2.4 Statistical method

Descriptive statistics were used to summarize the basic characteristics of the groups under study. Continuous variables were presented as the mean, standard deviation and median (with minimum and maximum values). To assess differences between groups, A One-Way Analysis of Variance (ANOVA) was conducted. For further pairwise comparisons, either Tukey's Honestly Significant Difference (HSD) or Tamhane's T2 tests were applied. Statistical significance was set at a p-value of less than 0.05. All calculations were performed using IBM SPSS Statistics 22 (SPSS Inc., an IBM Co., Somers, New York, NY, USA).

3. Result

3.1 Biochemical analyses

In Group 2, the TOS level was measured at 19.75 ± 0.75, which was significantly higher compared to the other groups ($p < 0.001$). In Group 3, TOS levels showed a significant decrease, with a value of 14.82 ± 0.96 ($p < 0.001$) (Table 1). Similarly, MDA levels in Group 2 were significantly higher compared to Group 1 ($p = 0.010$). However, in Group 3, MDA levels demonstrated a statistically significant reduction compared to Group 2, with a value of 7.83 ± 1.22 ($p = 0.043$) (Table 2).

In Group 2, SOD and GSH-Px activities were suppressed compared to Group 1, with values of 21.77 ± 2.57 and 3.97 ± 0.65, respectively ($p = 0.024$ and $p = 0.043$). In contrast, Group 3 exhibited a significant increase in the levels of both antioxidant enzymes ($p = 0.036$ and $p = 0.034$, respectively) (Table 2). TAS levels in Group 2 were the lowest among all the groups, at 0.85 ± 0.05 ($p < 0.001$). In Group 3, TAS levels showed a significant increase compared to Group 2, measured at 1.71 ± 0.15 ($p < 0.001$) (Table 1).

3.2 Histopathological evaluation

Microscopic examinations revealed that desquamation, edema, inflammation and vasocongestion scores were significantly lower in Group 1 compared to the other groups ($p < 0.001$).

TABLE 1. Comparison of TAS and TOS values obtained from serum.

Groups	Mean ± SD	Median (Min–Max)	*p*-Values	*Post Hoc p*-Values
TAS (mmoL/L of Trolox equivalent)				
1	1.92 ± 0.09	1.95 (1.77–2.01)		1–2: <0.001*
2	0.85 ± 0.05	0.85 (0.78–0.90)	<0.001*	1–3: 0.010*
3	1.71 ± 0.15	1.66 (1.55–1.99)		2–3: <0.001*
TOS (mmoL/L Trolox equivalent)				
1	11.88 ± 0.82	11.83 (11.10–13.30)		1–2: <0.001*
2	19.75 ± 0.75	19.90 (18.40–20.60)	<0.001*	1–3: <0.001*
3	14.82 ± 0.96	14.70 (13.90–16.10)		2–3: <0.001*

*Note: TAS: Total antioxidant status; TOS: Total oxidant status; SD: Standard deviation; Min: Minimum; Max: Maximum; *: Significantly.*

TABLE 2. Comparison of SOD, GSH-px, MDA values obtained from corporal tissue.

Groups	Mean ± SD	Median (Min–Max)	*p*-Values	*Post Hoc p*-Values
SOD (U/g protein)				
1	62.90 ± 23.75	51.30 (43.60–97.90)		1–2: 0.024*
2	21.77 ± 2.57	21.10 (18.80–25.50)	0.002*	1–3: 0.391
3	44.88 ± 14.90	44.40 (28.50–69.20)		2–3: 0.036*
GSH-Px (U/g protein)				
1	10.79 ± 4.59	8.49 (7.98–19.64)		1–2: 0.043*
2	3.97 ± 0.65	3.99 (3.01–4.98)	0.003*	1–3: 0.234
3	6.44 ± 1.62	5.99 (4.73–8.65)		2–3: 0.034*
MDA (μmoL/g wet tissue)				
1	6.99 ± 1.58	6.91 (4.82–8.99)		1–2: 0.010*
2	10.81 ± 2.67	11.39 (6.98–13.42)	0.009*	1–3: 0.732
3	7.83 ± 1.22	7.67 (6.54–9.87)		2–3: 0.043*

*Note: SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; MDA: Malondialdehyde; SD: Standard deviation; Min: Minimum; Max: Maximum; *: Significantly.*

In Group 2, edema and vasocongestion scores were markedly increased compared to Group 1 ($p < 0.001$). In Group 3, both edema and vasocongestion scores were observed to decrease compared to Group 2, with values of 1.07 ± 0.29 and 1.08 ± 0.13, respectively ($p < 0.001$). Similarly, the level of desquamation in Group 3 showed significant improvement compared to Group 2 ($p < 0.001$). On the other hand, the inflammation score in Group 2 was significantly higher than in the other groups, measured at 2.02 ± 0.1. In Group 3, inflammation was markedly suppressed compared to Group 2 ($p < 0.001$) (Fig. 1, Table 3).

4. Discussion

Priapism is a disorder of the mechanisms that regulate penile detumescence and erection [2]. Ischemic priapism is the most prevalent form, with an overall incidence of 1.5 cases per 100,000 person-years [19]. It develops as a result of venous outflow obstruction in the corpus cavernosum, which subsequently impedes arterial inflow. Without prompt treatment, corporal ischemia and necrosis become inevitable [1, 2]. The cornerstone of treatment includes corporal aspiration and the administration of intracavernous alpha-adrenergic agents. In refractory cases, shunt surgeries and penile prosthesis implantation are other modalities [19]. However, paradoxically, the restoration of arterial blood flow to the penile tissue results in ischemia-reperfusion injury. Minimizing this damage is crucial for preserving the optimal function of erectile tissue [9].

The pathophysiology of ischemia-reperfusion injury remains incompletely understood; however, numerous mechanisms have been identified. These processes involve a reduction in oxidative phosphorylation, a heightened requirement for adenosine triphosphate (ATP), increased levels of intracellular Ca^{2+} and Na^{+}, a decrease in intracellular pH, and the activation of proteases and phosphatases, along with various other pathophysiological mechanisms [9, 20]. Additionally, proinflammatory mediators, leukocyte infiltration and endothelial damage play significant roles in ischemia-reperfusion injury [4, 21]. As a result, there is a substantial increase in ROS in the environment. ROS initiate enzymatic reactions, such as the peroxidation of plasma lipoproteins or polyunsaturated fatty acids, leading to oxidative damage in cell membranes and an increased production of toxic metabolites [9, 22].

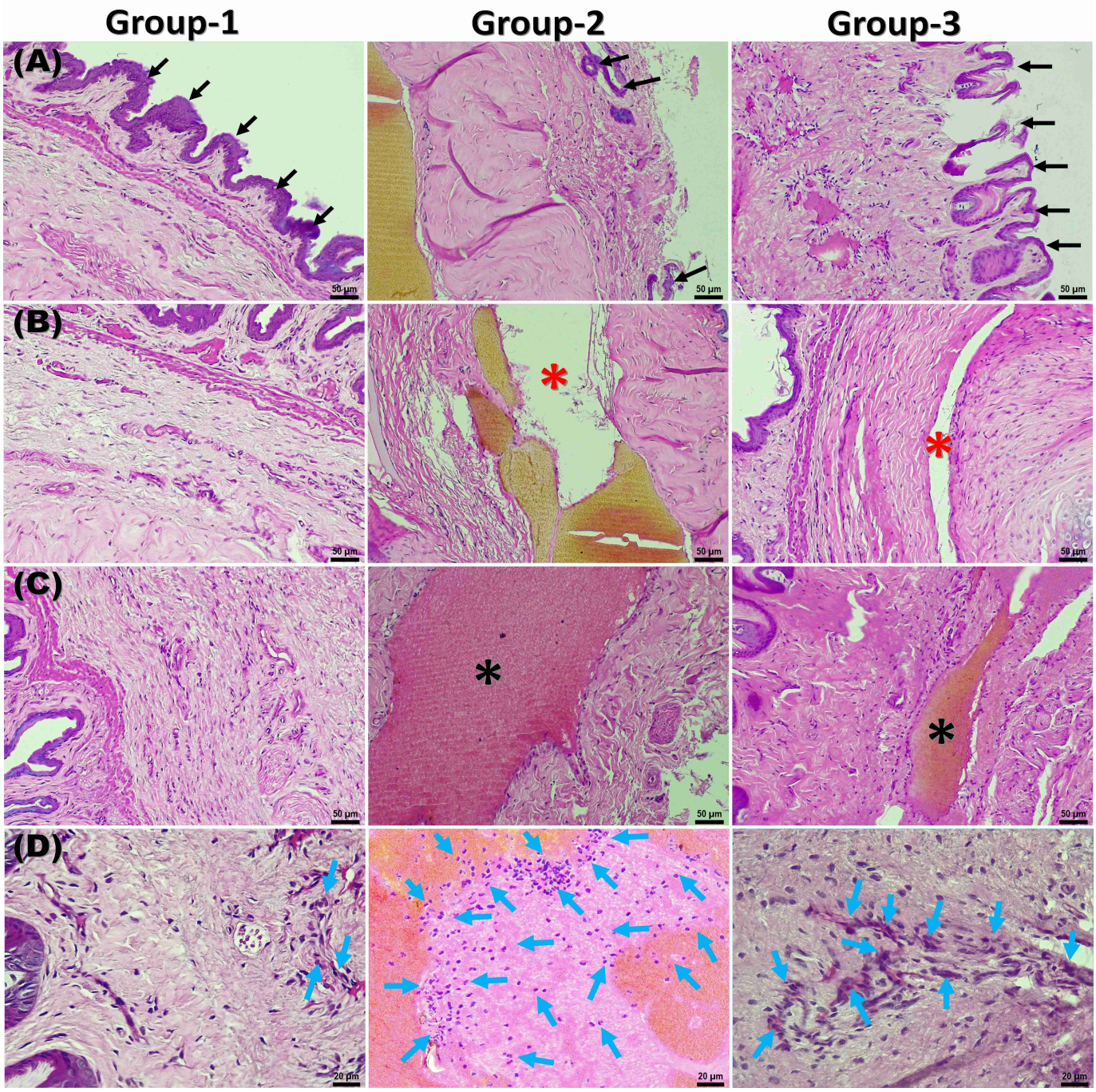

FIGURE 1. Representative microscopic images of hematoxylin-eosin-stained penile tissues from the study groups. (A) Evaluation of desquamation (black arrows: epithelium). In Group 1, the epithelium shows normal thickness. In Group 2, severe desquamation is observed, with very few areas showing intact epithelium. Group 3 shows reduced epithelial thickness with mild desquamation. (B) Comparison of edema (red stars: areas of edema). No edema is observed in Group 1. Group 2 shows marked and diffuse edema. Group 3 exhibits mild edema. (C) Comparison of vasocongestion and haemorrhage (black star: haemorrhagic and vasocongestion areas). Group 1 shows normal stromal areas with vascular structures of normal caliber. In Group 2, intense vasocongestion and severe hemorrhagic area are observed. Group 3 exhibits moderate vasocongestion and hemorrhagic areas. (D) Comparison of inflammation (blue arrow: areas of inflammatory cell infiltration). In Group 1, inflammatory cell infiltration is minimal to negligible. Group 2 displays areas of intense inflammatory cell infiltration. Group 3 shows mild to moderate levels of inflammatory cell infiltration. (Scale bars: 50 μm for A–C; 20 μm for D).

TABLE 3. Comparison of desquamation, edema, inflammation and vasocongestion scores in rat groups.

Groups	Mean ± SD	Median (Min–Max)	*p*-Values	*Post Hoc p*-Values
Desquamation				
1	0.17 ± 0.06	0.15 (0.13–0.31)		1–2: <0.001*
2	2.03 ± 0.25	1.95 (1.63–2.32)	<0.001*	1–3: <0.001*
3	0.91 ± 0.24	0.83 (0.61–1.30)		2–3: <0.001*
Edema				
1	0.21 ± 0.07	0.23 (0.12–0.31)		1–2: <0.001*
2	2.25 ± 0.31	2.09 (1.93–2.63)	<0.001*	1–3: <0.001*
3	1.07 ± 0.29	1.09 (0.50–1.43)		2–3: <0.001*
Inflammation				
1	0.14 ± 0.06	0.13 (0.08–0.26)		1–2: <0.001*
2	2.02 ± 0.10	2.00 (1.94–2.24)	<0.001*	1–3: <0.001*
3	0.99 ± 0.09	1.00 (0.88–1.09)		2–3: <0.001*
Vasocongestion				
1	0.24 ± 0.07	0.27 (0.13–0.32)		1–2: <0.001*
2	2.31 ± 0.19	2.22 (2.11–2.60)	<0.001*	1–3: <0.001*
3	1.08 ± 0.13	1.03 (0.90–1.31)		2–3: <0.001*

Note: Desquamation, edema, inflammation, vasocongestion.
*SD: Standard deviation; Min: Minimum; Max: Maximum; *: Significantly.*

Sugammadex is a hydrophilic molecule with a lipophilic core and is the first specific agent used to rapidly reverse neuromuscular blockade induced by vecuronium or rocuronium [23]. It has a half-life of approximately 2 hours and is excreted via the urinary system, largely unchanged [24]. Sugammadex is widely used in anesthesia practice for postoperative extubation and for reversing newly developed blocks in patients who cannot be intubated or ventilated [25, 26]. Additionally, recent studies have shown that sugammadex is an effective pharmacological agent in preventing the destructive effects of oxidative stress in ischemia-reperfusion models conducted on various tissues [5, 6].

Kadioglu *et al*. [6] documented in their experimental study on an ovarian ischemia-reperfusion model that sugammadex reduced oxidative damage by inhibiting polymorphonuclear leukocyte infiltration in ovarian tissue. The same study also reported an increase in antioxidant enzyme activities such as SOD, catalase and GSH. In alignment with these findings, our research demonstrated a marked increase in antioxidant enzyme activity and total antioxidant capacity within the treatment group. Similarly, an experimental study by Alagöz *et al*. [5] documented that sugammadex helped mitigate the damaging effects of ischemia-reperfusion injury in the lower extremities by reducing inflammation. In another study, Yeşiltaş *et al*. [27] reported that sugammadex alleviated allergic inflammatory changes in the lungs of rats caused by rocuronium. Similarly, Koç *et al*. [4] demonstrated that sugammadex is a beneficial molecule in preventing oxidative stress by reducing levels of pro-inflammatory cytokines such as interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) in a gastric ischemia-reperfusion model in rats. In our study, histopathological evaluations revealed a significant reduction in inflammation scores in rats treated with sugammadex. Furthermore, a review of the literature indicates that sugammadex has been shown to exhibit protective effects against oxidative damage in vital organs, including the brain and kidneys [7, 8].

In our study, the histopathological examination of penile tissue revealed that sugammadex significantly improved tissue damage parameters such as edema, desquamation and vasocongestion. Additionally, it was observed to markedly improve TOS levels. In another study, Doğan *et al*. [28] reported that rocuronium caused oxidative damage in lung tissue and sugammadex suppressed the harmful effects by reducing the number of mast cells in the lung tissue. A recent study also evaluated the effect of sugammadex on glutamate-induced cytotoxicity, finding that this pharmacological agent significantly decreased, neuronal nitric oxide synthase, nitric oxide, TOS levels and apoptotic cells count, while increasing TAS levels. The authors concluded that sugammadex may have positive effects on cytotoxicity in neurodegenerative diseases [29]. Although apoptotic cell counts could not be determined in our study, we observed a significant decrease in MDA levels, a marker of lipid peroxidation, in the corporal tissue after sugammadex treatment. We believe that this reduction in MDA levels may provide a protective role against penile ischemia reperfusion injury and potentially contributing to sexual life after priapism treatment.

A significant limitation of our study is the inability to measure pro-inflammatory cytokine levels and protein degradation products in blood biochemical analyses. Additionally, only hematoxylin-eosin staining was used in histopathological evaluations, and immunohistochemical staining methods were not applied. Another limitation is that our study focused solely on

the early effects of sugammadex treatment, without analyzing its long-term effects.

5. Conclusions

This experimental study provides both biochemical and histopathological evidence that sugammadex, a widely used agent in anesthesia practice for reversing the effects of muscle relaxants, demonstrates a protective activity in preventing oxidative stress in erectile tissues. Our findings necessitate further validation through comprehensive experimental and randomized clinical studies in both andrology and anesthesia practices.

ABBREVIATIONS

ROS, reactive oxygen species; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; TOS, total oxidant status; TAS, total antioxidant status; ATP, adenosine triphosphate; ANOVA, a one-way analysis of variance; HCL, hydrogen chloride; HSD, honestly significant difference; IL, interleukin; TNF, tumor necrosis factor.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

VK, KY and AE—designed the research study; wrote the manuscript. VK, KY, AE, FG, ABG and VU—performed the research. VK, FG, ABG and VU—analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animal study protocol was approved by Tokat Gaziosmanpaşa University Ethics Committee for Experimental Animal (Decision No: 2024-HADYEK-12, Date: 07 August 2024). The rats were conducted in compliance with animal welfare guidelines of the National Institutes of Health.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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