https://doi.org/10.52973/rcfcv-e35554 Revista Científica, FCV-LUZ / Vol. XXXV

Effect of Cortexin administration on Kisspeptin and Spexin expression after testicular torsion

Efecto de la administración de Cortexina en la expresión de Kisspeptina y Spexina tras la torsión testicular

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ABSTRACT

Kisspeptin–1 (KISS–1) and Spexin (SPX) are neuropeptides that play crucial roles in metabolism and sexual function, with their expression levels in tissues potentially influenced by antioxidant treatments. This study aimed to investigate the effects of cortexin treatment against ischemia–reperfusion injury (I/R) resulting from testicular torsion on KISS–1 and SPX levels in testicular tissues. Twenty–eight male Sprague–Dawley, rats, aged 8–10 weeks, were divided into four equal groups: control, torsion, torsion/detorsion, and torsion/detorsion+cortexin. At the conclusion of the experiment, histopathological and immunohistochemical analyses were performed to assess the expressions of KISS–1, SPX, tumor necrosis factor–alpha (TNF–α), and Caspase–3 in the testicular tissues. For biochemical analyses, total antioxidant status (TAS) and total oxidant status (TOS) levels were measured in serum samples using the ELISA method, while malondialdehyde (MDA) levels were assessed spectrophotometrically in testicular tissues. The results showed that compared to the control group, the torsion and torsion/detorsion groups exhibited significant histopathological damage, along with increased levels of MDA, TOS, Caspase–3, and TNF–α, and decreased levels of TAS, KISS–1, and SPX in the testicular tissues. Conversely, in the torsion+detorsion+cortexin group, which received treatment for reperfusion injury, there was a notable reduction in tissue damage, with decreased levels of MDA, TOS, caspase–3, and TNF–α, alongside increased levels of TAS, KISS, and SPX. Cortexin decreases testicular damage by reducing oxidative stress, increases spermatogenesis by improving seminiferous tubule and germinal epithelial thickness, and regulates KISS–1 and SPX expression, which have effects on the reproductive system.

Key words: Kisspeptin–1, Spexin, Testicular damage, Torsion, Detorsion

RESUMEN

La kisspeptina (KISS) y la spexina (SPX) son neuropéptidos que desempeñan papeles cruciales en el metabolismo y la función sexual, y sus niveles de expresión en los tejidos pueden verse influidos por tratamientos antioxidantes. El objetivo de este estudio fue investigar los efectos del tratamiento con cortexina contra la lesión por isquemia–reperfusión (I/R) resultante de la torsión testicular sobre los niveles de KISS y SPX en el tejido testicular. Veintiocho ratas albinas Wistar macho, de 8–10 semanas de edad, se dividieron en cuatro grupos iguales: control, torsión, torsión/detorsión y torsión/detorsión+cortexina. Al finalizar el experimento, se realizaron análisis histopatológicos e inmunohistoquímicos para evaluar las expresiones de KISS, SPX, factor de necrosis tumoral alfa (TNF–α) y caspasa–3 en los tejidos testiculares. Para los análisis bioquímicos, se midieron los niveles de estado antioxidante total (TAS) y estado oxidante total (TOS) en muestras de suero mediante el método ELISA, mientras que los niveles de malondialdehído [MDA] se evaluaron espectrofotométricamente en tejidos testiculares. Los resultados mostraron que, en comparación con el grupo de control, los grupos de torsión y torsión/detorsión presentaban un daño histopatológico significativo, junto con un aumento de los niveles de MDA, TOS, caspasa–3 y TNF–α, y una disminución de los niveles de TAS, KISS y SPX en los tejidos testiculares. Por el contrario, en el grupo de torsión+detorsión+cortexina, que recibió tratamiento para la lesión por reperfusión, se produjo una notable reducción del daño testicular, con una disminución de los niveles de MDA, TOS, caspasa–3 y TNF–α, junto con un aumento de los niveles de TAS, KISS y SPX. La cortexina disminuye el daño testicular reduciendo el estrés oxidativo y regula la expresión de kisspeptina y spexina

Palabras clave: Kisspeptina; spexina; testículo; torsión; detorsión

Received: 30/10/2024 Accepted: 09/03/2025 Published: 28/04/2025

INTRODUCTION

Testicular torsion is a common urological condition in which the spermatic cord supplying the testicles rotates around its longitudinal axis. Following detorsion, testicular torsion can lead to reperfusion injury [1]. Rapid diagnosis and surgical intervention are crucial to prevent ischemic damage and subsequent loss of germ cells [2]. Histological and molecular damage after testicular torsion/detorsion–induced reperfusion is primarily caused by the increase in reactive oxygen species [ROS] and neutrophil infiltration that occurs shortly after reperfusion [3]. Experimental studies highlight the importance of using various agents to prevent damage that may occur after detorsion by suppressing oxidative stress, inflammation, or apoptosis [4, 5, 6].

Cortexin is a polypeptide consisting of 82 amino acids obtained from the cerebral cortex of cattle and pigs [7, 8]. It is also reported that it shows antioxidant activity by reducing lipid peroxidation and has anti–apoptotic character [9, 10]. Ischemia reperfusion injury affects many pathological cellular processes, including reactive oxygen species [11].

Kisspeptin–1 (KISS–1) and Spexin (SPX) are closely related neuropeptides that play crucial roles in regulating metabolism, body weight, and sexual function [12]. KISS–1 is encoded by the KISS–1 gene and plays a significant role in reproductive processes by modulating the hypothalamic–pituitary–gonadal axis [13]. KISS interacts with the KISS1 receptor, promoting the secretion of gonadotropin–releasing hormone (GnRH). This interaction is vital for the regulation of Leydig cells and spermatogenesis [14].

SPX, also known as neuropeptide Q, is the most recently discovered member of the galanin/kisspeptin family. It plays a pivotal role in various physiological processes, including energy metabolism, obesity, diabetes, gastrointestinal motility, as well as cardiovascular and renal functions [15, 16]. Additionally, it is also involved in the regulation of gonadotropins [16, 17]. Although studies on spexin (SPX) in the testis are limited, it has been reported that SPX, which is involved in the maintenance of reproductive function, is strongly expressed in Leydig cells in rats [18].

There is significant interest in developing novel therapeutic agents that can suppress the onset and progression of testicular torsion. Accordingly, the present study aimed to investigate the potential protective effect of cortexin against inflammation and oxidative stress in testicular damage. Additionally, we sought to assess the effect of cortexin on modulating the expression of KISS–1 and SPX in testicular tissue.

MATERIALS AND METHODS

Surgical procedure and Experimental design

Animals were monitored under a 12 h light (07:00–19:00) and 12 h darkness (19:00–07:00) cycle, with the ambient temperature maintained at a constant range of 22–25°C. All animals were provided standard laboratory chow ad libitum, allowing unrestricted access to both water and food throughout the study.The doses and duration of anesthetic agents used during surgical procedures ketamine50 mg·kg^{-1} ketamine (Alfamine; Alfasan IBV, Woerden, The Netherlands), and 10 mg·kg^{-1} xylazine (Alfazine; Alfasan IBV, Woerden, The Netherlands) were determined as previously described [19]. The anesthesia dose was repeated every half hour. The right testicles of anesthetized rats were exposed and dissected through a longitudinal scrotal incision. Torsion of the right testicle was then performed by rotating it 720° counterclockwise and securing it to the scrotum for 1 h using three 6/0 silk sutures. In the torsion/detorsion group, testicular torsion was maintained for 1 h, followed by detorsion for 4 h, with all experimental groups being monitored for a total of 24 h.

In this study, 10 to 12 week–old male Sprague–Dawley rats (*Rattus norvegicus*) weighing (Balance equipment: TEMS, gts 30, Türkiye) between 340–360 g were divided into four groups, each consisting of 7 animals.Control group (n=7): To determine the possible effect of surgical stress on the testicles, the right testicles of the rats in this group were removed and then placed back into the scrotum without torsion. Torsion group (n=7): Torsion was performed for one h. Torsion/Detorsion group (n=7): Torsion was induced for one hour, followed by reperfusion with detorsion for 4 hours as previously described [20]. Torsion/Detorsion+Cortexin group (n=7): After one hour of torsion, 5 mg·kg^{-1} cortexin (Koptekcnh; Geropharm pharmaceutical company, Saint Petersburg, Russia) was administered intraperitoneally (i.p.) simultaneously with detorsion and reperfusion was sustained for 4 h.

Tissue and serum collection from experimental groups

At the end of the experiment, intracardiac blood was collected from the anesthetized rats, and testicular tissues were excised. For biochemical analyses, blood samples were placed in serum separation tubes; after coagulation, they were centrifuged at 1107 g for 10 min (Hettich ROTİNA 380/380R, Germany). Some testicular tissue was washed in physiological saline for malondialdehyde (MDA) analysis and then stored with serum samples at -80°C (NÜVE–DF 290 Türkiye) until further analysis. For histological evaluation, tissues were preserved in 10% Neutral formaldehyde solution at room temperature.

Tissue preparation

Following fixation in 10% formalin solution, tissue sections with a thickness of 4–6 µm were obtained from paraffin blocks using a Thermo Shandon Finesse ME microtome (Thermo Fisher Scientific, Cheshire, UK). The paraffin blocks were prepared through routine tissue processing using an automatic tissue processor (Leica TP1020, Nussloch, Germany). Subsequently, the sections were stained using standard Hematoxylin–Eosin (H&E) staining and evaluated semi–quantitatively under a Leica DM500 microscope.

Histopathological evaluations

Tissue samples were stained using the standard H&E staining technique to examine structural changes. Histopathological alterations in all samples were analyzed and photographed using a light microscope (Leica DM500 Nussloch, Germany) y two blinded histologists. To assess the degree of histopathological changes in the testes, 20 different histological fields from each group were evaluated semi–quantitatively at 20× magnification. The semi–quantitative analyses of pathological changes were scored as none (0), mild (1), moderate (2), and severe (3). The features examined included vacuolization of the seminiferous tubule epithelium,

edema, vascular congestion, the presence of apoptotic cells in the seminiferous tubule lumen, and the structure of Leydig cells in the interstitial area.

Histological evaluation and maturation of seminiferous tubules

For light microscopic evaluations, the diameter of seminiferous tubules and the thickness of the germinal epithelium were measured in 30 randomly selected seminiferous tubules at 10× magnification in each section [21]. Modified Johnsen scoring was employed to evaluate spermatogenesis in the same seminiferous tubules. Spermatogenic cells were examined using a Leica DM500 microscope and assessed based on maturation and density, utilizing a scoring table that assigned scores ranging from 1 to 10 (TABLE I) [22].

TABLE I
The modified Johnsen scoring for evaluation of spermatogenesis

10: Complete spermatogenesis with mature sperm cells
9: There are few sperm cells with disorganized germinal epithelium
8: There are less than 10 sperm cells (less than 5–10)
7: There are no sperm cells, there are spermatids
6: No sperm cells, less than 10 spermatids (less than 5–10)
5: There are no sperm cells and spermatids, there are spermatocytes
4: No sperm cells and spermatids, less than 5 spermatocytes
3: There are only spermatogonia as germ cells
2: There are no germ cells, only Sertoli cells
1: There are no cells in the seminiferous tubule

Immunohistochemical analysis

Sections obtained from paraffin blocks onto polylysine slides were deparaffinized and passed through a graded alcohol series, followed by boiling in a microwave oven(Vestel AMD–2011 × 1000 W. Türkiye), (750W) in citrate buffer solution (pH 6) for 12 min. The sections were then washed with phosphate–buffered saline (PBS) and treated with hydrogen peroxide solution for 6 min. After washing with PBS for three sets of 5 min, a blocking solution was applied for 5 min.

Subsequently, the sections were incubated with anti–kisspeptin antibody (1:200; Abcam, EPR23770-189, ab275874, London, UK), anti–neuropeptide Q2/NPQ2 SPX antibody (1:200; A04088, Booster Biological Technology, Pleasanton, CA, USA), caspase–3 antibody (1:200; Abcam, ERP18297–ab184787, UK), and TNF–α antibody (1:200; Abcam, ab220210, London, UK) for 60 min in a humidified environment at room temperature. After washing with PBS, a secondary antibody compatible with the primary antibodies was applied to the sections for 30 min in a humid environment.

The sections were then incubated with streptavidin peroxidase (TS–125–HR, Lab Vision Corporation, USA) for 30 min at room temperature and placed in PBS. Following this, a solution of 3–amino–9–ethylcarbazole (AEC) substrate and AEC chromogen was added, and images were captured using a light microscope, with all groups washed with PBS simultaneously. The sections were counterstained with Mayer's hematoxylin and covered with PBS and distilled water. The prepared sections were examined and photographed under a Leica DM500 microscope. The prevalence and severity of immunoreactivity were determined as follows: 0.1 for <25%, 0.4 for 26–50%, 0.6 for 51–75%, 0.9 for 76–100%, and 0 for none; +0.5 for weak, +1 for moderate, and + for severe, as previously described [23].

Biochemical Analysis

Determination of MDA levels

A buffer solution for analysis was prepared by combining 0.42 g of Tris–Base, 1.43 g of Tris–HCl, 3 g of KCl, and 0.5 mL of Tween 20 in 250 mL of distilled water. Tissue samples were homogenized in this buffer solution. The resulting homogenate was then centrifuged at 16000 rpm for 4 min, and 1 mL of the supernatant was transferred into another tube. To this, 1 mL of 10% TCA, 1 mL of 0.6% TBA, 1 mL of distilled deionized water (ddH2O), and 0.5 mL of 4% HCl were added, followed by incubation at 90–95°C for 120 min. After incubation, the tubes were cooled at room temperature for 30 min. Subsequently, 3 mL of butanol was added to each tube, and the mixture was centrifuged at 5,000 rpm for 5 min. The supernatant was then assayed at 532 nm using a spectrophotometer(UV–1201V–Shımadzu Japan) [24].

Total Antioxidant and Oxidant Status Analysis

TAS and TOS levels in serum samples were measured according to the procedures specified by the manufacturer (Rel Assay Kit Diagnostics, Türkiye). An automatic plate washer (BioTek ELX50, BioTek Instruments, USA) was used for plate washing, and absorbance readings were obtained using the ChroMate Microplate Reader P4300 (Awareness Technology Instruments, USA). Results for TAS and TOS levels are expressed as mmol Trolox and H_2O_2 equivalent per liter, and μmol H_2O_2 eq·L^{-1} respectively.

Statistical Analysis

Statistical analysis was performed using SPSS version 22 software. Numerical measurements were summarized using median and minimum–maximum values. The Kruskal–Wallis test was employed for overall comparisons among more than two groups, while the Mann–Whitney U test was used for pairwise comparisons between two groups. Additionally, Bonferroni correction was applied, setting the statistical significance level at 0.01 for all tests. For the analysis of dependent variables, a paired samples t–test was conducted with a significance level of 0.05. And also GraphPad Prism 8.4 was used to make a graphic presentation of the data collected during the investigation.

RESULTS AND DISCUSIONS

In this study, it was demonstrated that cortexin, when administered in response to ischemia–reperfusion (I/R) injury, reduced histopathological damage in testicular tissue, increased antioxidant levels, and enhanced KISS–1 and SPX expressions. The primary causes of testicular tissue damage under ischemic conditions include excessive reactive oxygen species (ROS) production, inflammation, and cellular apoptosis, resulting from

the oxidation of cell membrane lipids, proteins, and DNA [3, 25]. Testicular torsion induces histopathological changes due to increased oxidative stress and I/R–induced cellular damage.

Histopathological results of the presented study showed that control group (FIG. 1.1A–1D), H&E staining revealed normal testicular histology with organized seminiferous tubules, intact Sertoli cells, and Leydig cells. In the torsion group (FIG. 1.2A–2D), significant increases in apoptotic cells, vacuolated germinal epithelium, edema, and vascular congestion were observed (P<0.05). Apoptotic Leydig cells in the seminiferous tubule lumen showed no statistical significance (P>0.05). In the torsion + detorsion group (FIG. 1.3A–3D), apoptotic cells, vacuolated epithelium, edema, vascular congestion, and apoptotic Leydig cells were significantly higher compared to the torsion group (P<0.05). The torsion + detorsion + cortexin group (FIG. 1.4A–4D) demonstrated significant reductions in apoptotic cells, vacuolated epithelium, edema, vascular congestion, and apoptotic Leydig cells compared to the torsion + detorsion group (P<0.05) (TABLE II). The epithelial thickness of seminiferous tubules in the torsion and torsion+detorsion groups was significantly decreased compared to the control group (P<0.05). However, the torsion+detorsion+cortexin group showed a significant increase in epithelial thickness compared to the torsion+detorsion group (P<0.05)(FIG. 2A). Seminiferous tubule diameters were similar in the Control and torsion+detorsion+cortexin groups (P=0.537), but significantly decreased in the torsion and torsion+detorsion groups (P<0.05). The torsion+detorsion+cortexin group showed

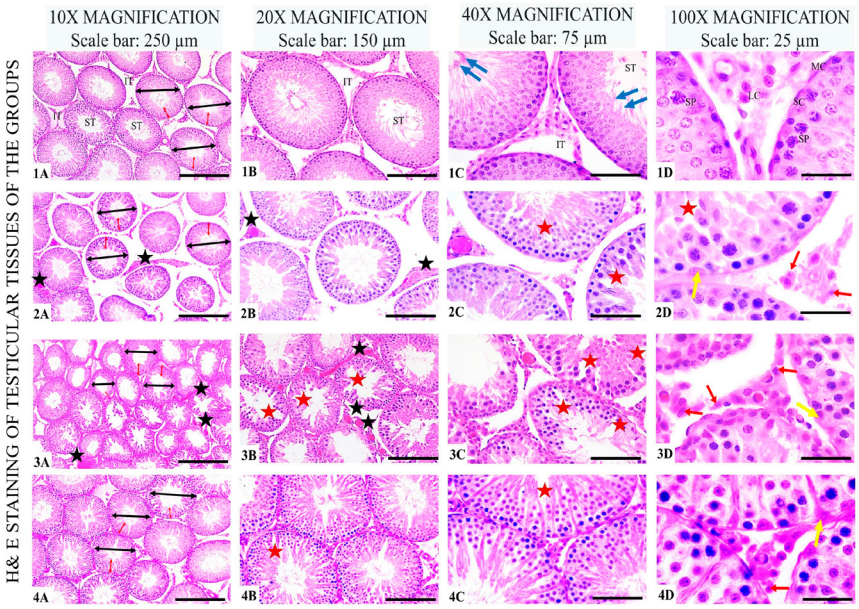


FIGURE 1. (a): Testicular tissues from rats in all groups were stained with H&E and photographed at different magnifications. (ST: seminiferous tubule, IT: interstitial tissue, MC: myoid cell, LC: Leydig cell, SP: spermatogenic cells, SC: Sertoli cell, Control: 1A–1D, Torsion: 2A–2D, Torsion+detorsion: 3A–3D, Torsion+detorsion+cortexin: 4A–4D). The control group and cortexin group show measurements of tubule diameter (double–headed black arrow) and germinal epithelium (double–headed red arrow) were similar, with normal cell structures observed in the seminiferous tubules and interstitial area, and spermatids and spermatozoa (blue arrow) densely observed in the lumen. Compared to the control group, the torsion and torsion + detorsion groups exhibited a reduction in tubule diameter and a decrease in germinal epithelium height, with congestion and edema (black star) in the interstitial area. Additionally, seminiferous tubules characterized by vacuolated germinal epithelium (red star), apoptotic Leydig interstitial cells (red arrow), and apoptotic Sertoli cells (yellow arrow) were observed. The torsion + detorsion + cortexin treatment group showed a reduction in tissue and cell damage compared to the torsion + detorsion and torsion groups.

TABLE II
Histological and morphometric analysis of testicular tissues in rats with testicular torsion either treated or not with cortexin

Groups	Vacuolization of seminiferous tubule epithelium		Congestion		Edema		Apoptotic cells in seminiferous tubule lumen		Structure of Leydig cells	
	Median (mean–max)	*P* value	Median (mean–max)	*P* value	Median (mean–max)	*P* value	Median (mean–max)	*P* value	Median (mean–max)	*P* value
Control	0.00(0.00-0.00)	<0.05	0.00(0,00-0.00)	<0.05	0.00(0.00-0.00)	<0.05	0.00(0.00-0.00)	<0.05	0.00(0.00-0.10)	<0.05
Torsion	0.40(0,20-0.85)[a]		0.55(0.32-0.80)[a]		0.33(0.20-0.44)[a]		0.50(0,37-0.68)[a]		0.27(0.14-0.41)[a]	
Torsion/Detorsion	1.58(1.25-2.40)[b]		1.78(1.29-2.48)[b]		1.69(1.23-2.88)[b]		1.95(1.78-2.28)[b]		1.44(1.25-2.25)[b]	
Torsion/Detorsion + Cortexin	0.32(0,25-3.70)		0.48(0.21-0.80)		0.31(0,15-0.69)		0.38(0.17-89)		0.50(0.15-0.75)	

The statistical scores of the groups based on the histopathological evaluations of testicular tissues are provided. Values are presented as median (min–max). [a]Compared to the control group. [b]Compared to the torsion group (P<0.05)

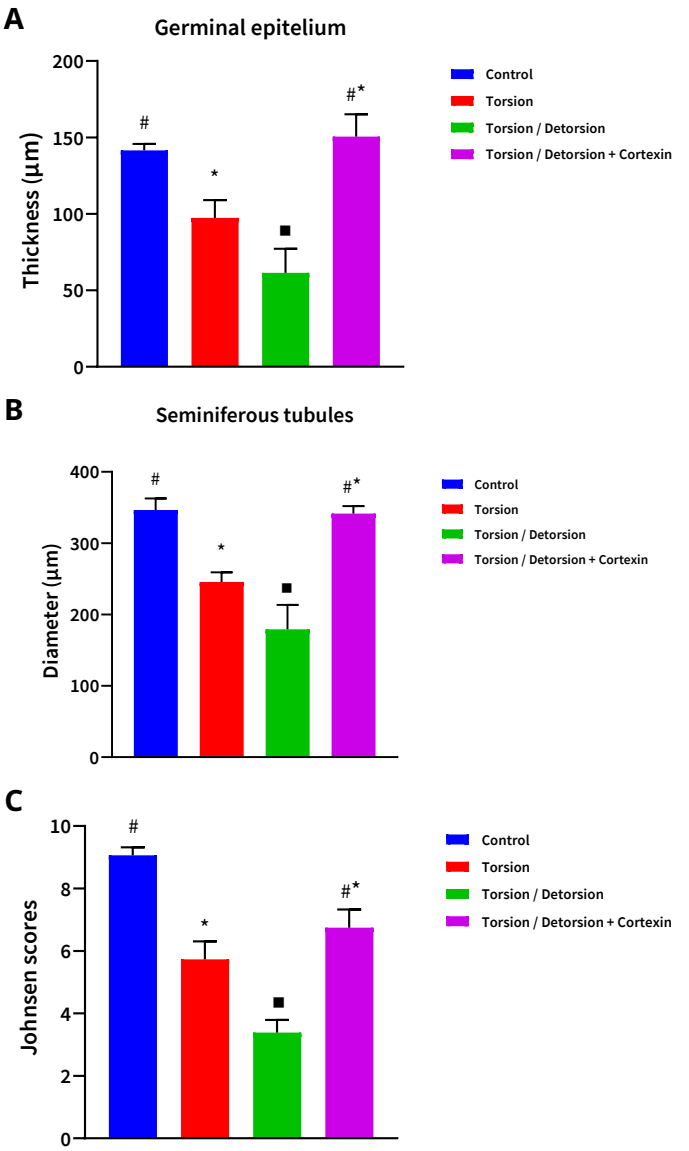
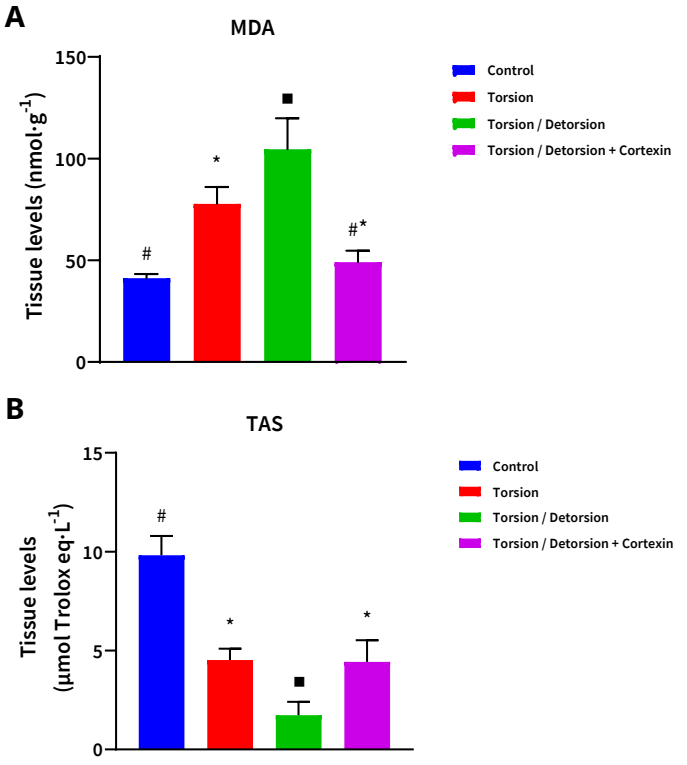


FIGURE 2. A: The thickness of the seminiferous tubule epithelium in the testicular tissues of rats (#; compared to the control group (*P*<0.05), * compared to the torsion group (*P*<0.05), ▪ compared to the torsion/detorsion group (*P*<0.05)). B: measurements of seminiferous tubule diameter in the testicular tissues of rats (#; compared to the control group (*P*<0.05), * compared to the torsion group (*P*<0.05), ▪ compared to the torsion/detorsion group (*P*<0.05)). C: Johnsen scores (#; compared to the control group (*P*<0.05), * compared to the torsion group (*P*<0.05), ▪ compared to the torsion/detorsion group (*P*<0.05))

a significant increase in tubule diameter compared to the other two groups (*P*<0.05) (FIG. 2B). Additionally, Johnsen scores were significantly lower in the torsion and torsion+detorsion groups compared to the control group (*P*<0.05), with further reduction in the torsion+detorsion group. Previous studies have reported similar histopathological findings in I/R injury in rat testicles [26, 27]. The torsion+detorsion+cortexin group showed a significant increase in Johnsen score compared to the torsion+detorsion group (*P*<0.05), but no significant difference compared to the torsion group (*P*=0.626) (FIG. 2C). Cortexin treatment in the torsion + detorsion group led to significant improvements in the histopathological outcomes. Specifically, it reduced apoptotic cells between basal spermatogonia and Sertoli cells, vacuolated appearance of the germinal epithelium and apoptotic Leydig cells in the interstitial area. In addition to Cortexin treatment significantly restored the epithelial thickness of seminiferous tubules, which was reduced in the torsion+detorsion group and significantly increased the seminiferous tubule diameters compared to the torsion and torsion+detorsion groups. In another result, Cortexin significantly improved spermatogenesis as indicated by the increased Johnsen score. Cortexin has been shown to be effective in cases of traumatic facial nerve paralysis and cisplatin–induced ototoxicity in other studies [28, 29]. however, no studies have been found regarding its effects on testicular tissue.

Biochemical results of the presented study showed that the torsion and torsion+detorsion groups had significantly higher MDA levels than the control group (*P*<0.05). The torsion+detorsion group had a lower MDA level compared to the torsion group (*P*<0.05). The MDA level in the torsion+detorsion+cortexin group was higher than in the torsion+detorsion group, but this difference was not statistically significant when compared to the torsion group (*P*=0.099) (FIG. 3A). The serum TAS level was significantly lower in the torsion, torsion+detorsion, and torsion+detorsion+cortexin groups compared to the control group (*P*<0.05), with the torsion+detorsion group showing a lower TAS level than the torsion group (*P*<0.05). However, the TAS level in the torsion+detorsion+cortexin group was not significantly different from the torsion group (*P*=0.927) (FIG. 3B).For TOS levels, no significant difference was found between the control and torsion+detorsion+cortexin groups (*P*=0.670). TOS levels were higher in the torsion and torsion+detorsion groups compared to the control group (*P*<0.05), and the torsion+detorsion group had higher levels than the torsion group (*P*<0.05). The TOS



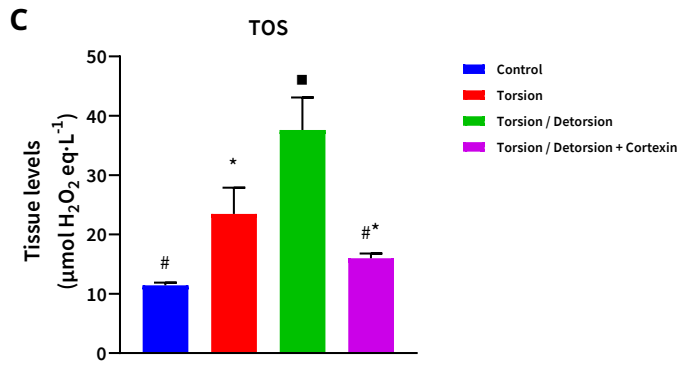


FIGURE 3. Effects of cortexin administration on antioxidant and oxidant parameters (MDA TAS and TOS results in experimental groups (#; compared to the (*P*<0.05), * compared to the torsion group (*P*<0.05), ▪ compared to the torsion/ detorsion group (*P*<0.05)

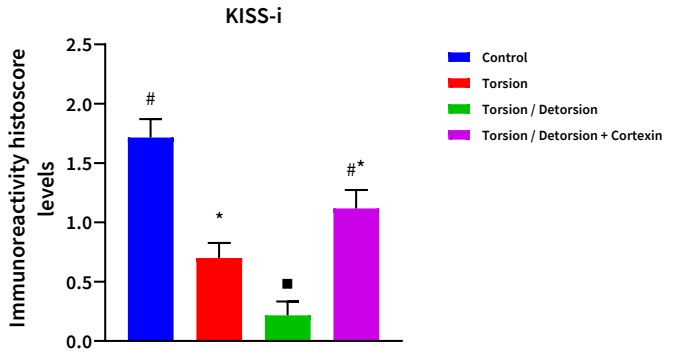


FIGURE 5. KISS–1 histoscore results in rat testicular tissue of all groups. (#; compared to the control group (*P*<0,05), * compared to the torsion group (*P*<0,05), ▪ compared to the torsion/detorsion group (*P*<0,05).

level in the torsion+detorsion+cortexin group was lower than in the torsion+detorsion group but did not differ significantly from the torsion group (*P*=0.541) (FIG. 3C). Although research on cortexin is limited, it is known for its strong antioxidant and tissue–specific anti–inflammatory effects [30]. In this study, MDA and TOS levels were elevated, while TAS levels were decreased in the torsion and detorsion groups compared to the control group. These findings indicate that I/R damage induces oxidative stress in the testicles, and cortexin mitigates this damage by reducing MDA and TOS levels and increasing TAS levels, consistent with prior research [31, 32]. Overall, cortexin seemed to have some influence on oxidative stress markers, but these changes were not always statistically significant.

Immunoreactivity results of presented study showed that KISS–1 expression was observed in the interstitial area of testicular tissue (FIG. 4: A–D). KISS–1 immunoreactivity was similar between the control and torsion+detorsion+cortexin groups (*P*=0.102), but significantly lower in the torsion and torsion+detorsion groups compared to the control (*P*<0.05). KISS–1 expression was also lower in the torsion+detorsion group compared to the torsion group (*P*<0.05). Although KISS–1 increased in the torsion+detorsion+cortexin group compared to the torsion+detorsion group, the difference from the torsion group was not significant (*P*=0.354)(FIG. 5). SPX expression was seen in both seminiferous tubules and the interstitial area (FIG. 6: A–D). SPX immunoreactivity was similar between the control and torsion+detorsion+cortexin groups (*P*=0.162), but significantly lower in the torsion and torsion+detorsion groups compared to the control (*P*<0.05). SPX expression was also lower in the torsion+detorsion group compared to the torsion group (*P*<0.05). Despite increased SPX in the torsion+detorsion+cortexin group compared to the torsion+detorsion group, the difference from the torsion group was not significant (*P*=0.149) (FIG. 7). This aligns with studies showing that testicular torsion/detorsion reduces GLP–1/ KISS expression [33], and disruption of the GLP–1–KISS–1-GnRH pathway is linked to testicular dysfunction in diabetic rats, with treatment improving KISS–1 expression and testicular structure [34]. SPX expression decreases under hypoxia, a potential ischemic trigger, suggesting a protective role for KISS and SPX in testicular

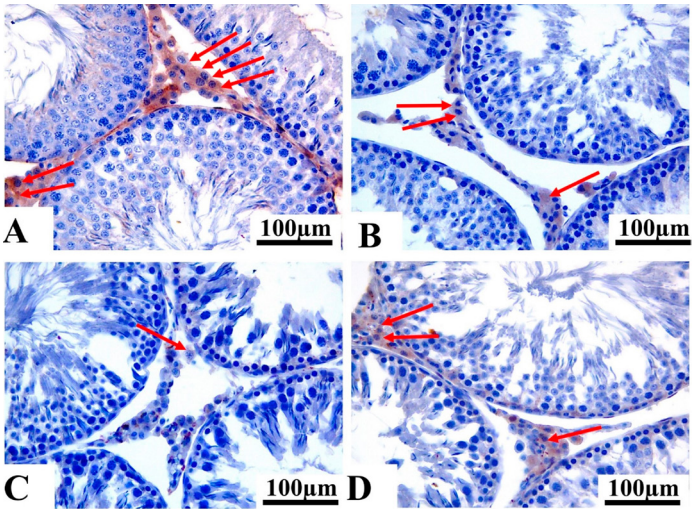


FIGURE 4. Immunoreactivity of KISS–1 in testicular tissue. (red arrow). A: Control, B: Torsion,C: Torsion + detorsion, D: Torsion+detorsion+Cortexin, Immunohistochemical staining, Mayers Hematoxylin, AEC chromogen scale bar: 100 µm

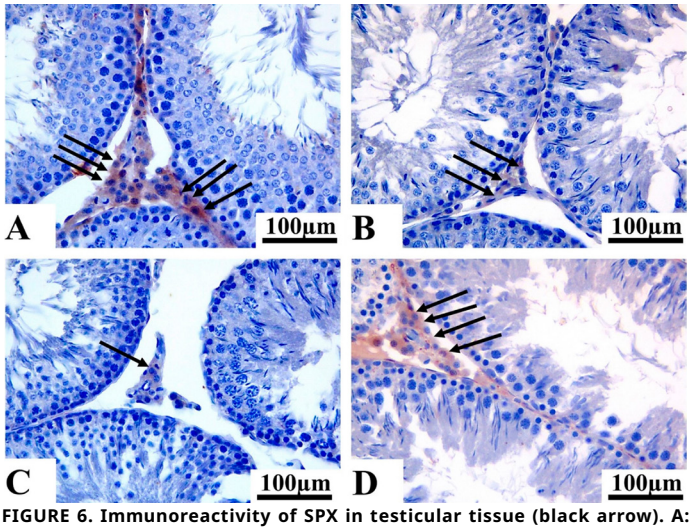


FIGURE 6. Immunoreactivity of SPX in testicular tissue (black arrow). A: Control, B: Torsion,C: Torsion+detorsion, D: Torsion+detorsion+cortexin, Immunohistochemical staining, Mayers Hematoxylin, AEC chromogen scale bar: 100 µm

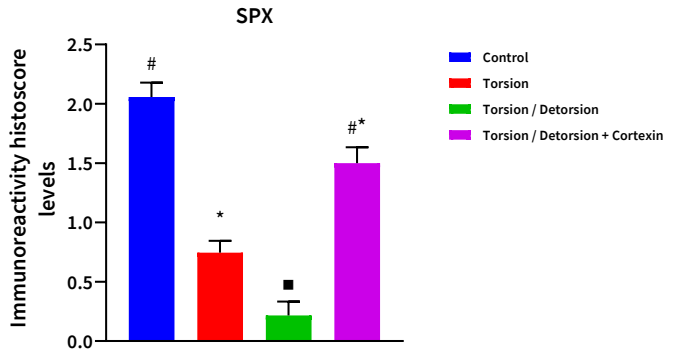


FIGURE 7: SPX histoscore results in rat testicular tissue of all groups. (#; compared to the control group (*P*<0.05), * compared to the torsion group (*P*<0.05), ▪ compared to the torsion/detorsion group (*P*<0.05)

dysfunction after torsion/detorsion [35]. Additionally, inflammation suppresses the reproductive axis, and treatments like adropin and SPX may reduce inflammation and tissue damage [36]. Long–term Olanzapine treatment reduced NPQ/SPX mRNA in the rat brainstem, and serum SPX levels dropped in acute myocardial infarction patients, suggesting a protective effect on cardiac metabolism [37, 38]. These findings suggest decreased SPX expression may result from reperfusion injury in the acute phase.

Results indicate that KISS–1 and SPX can be induced by torsion and detorsion, providing a protective mechanism alongside cortexin against testicular dysfunction. Both KISS–1 and SPX exhibit antioxidative and anti–apoptotic effects [39, 40].

Caspase–3 immunoreactivity was observed in both the interstitial area and the seminiferous tubules (FIG. 8:A–D). Caspase–3 expression was significantly increased in the torsion, torsion+detorsion, and torsion+detorsion+cortexin groups compared to the control group (*P*<0.05). When compared to the torsion group, Caspase–3 expression was higher in the torsion+detorsion group (*P*<0.05). However, although Caspase–3 expression in the torsion+detorsion+cortexin group was statistically lower compared to the torsion+detorsion group, the difference in Caspase–3 immunoreactivity compared to the torsion group was not statistically significant (*P*=0.754) (FIG. 9).

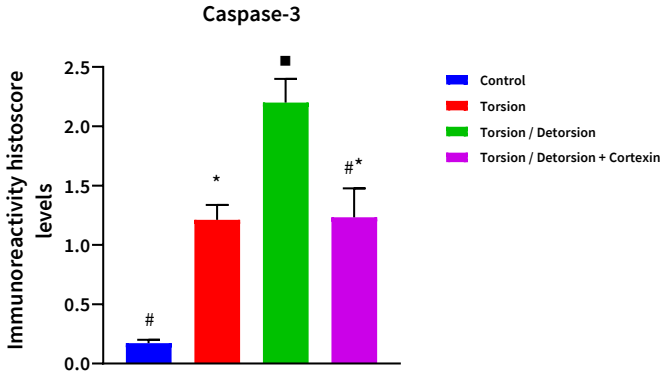


FIGURE 9. Caspase–3 histoscore results in rat testicular tissue of all groups. #; compared to the control group (*P*<0.05), * compared to the torsion group (*P*<0.05), ▪ compared to the torsion/detorsion group (*P*<0.05)

TNF–α immunoreactivity was observed in both the interstitial area and the seminiferous tubules (FIG. 10A–D). TNF–α expression increased in the torsion, torsion+detorsion, and torsion+detorsion+cortexin groups compared to the control group (*P*<0.05). When compared to the torsion group, TNF–α expression was higher in the torsion+detorsion group (*P*<0.05). However, in the torsion+detorsion+cortexin group, although TNF–α expression was statistically lower compared to the torsion+detorsion group, the decrease in TNF–α immunoreactivity compared to the torsion group was not statistically significant (*P*=0.791) (FIG. 11). Cortexin slightly reduced Caspase–3 and TNF–α expression in the torsion+detorsion

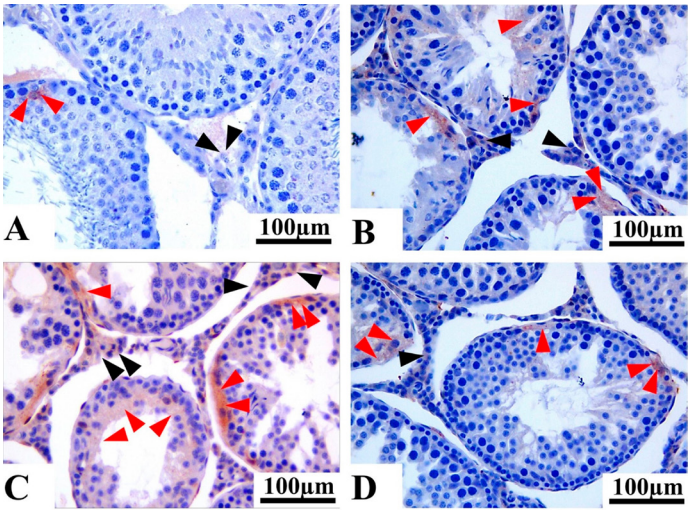


FIGURE 8. Immunoreactivity of Caspase–3 in testicular tissue (red arrowhead, black arrowhead). A: Control, B: Torsion,C: Torsion + detorsion, D: Torsion +detorsion+cortexin, Immunohistochemical staining, Mayers Hematoxylin, AEC chromogen scale bar: 100 µm

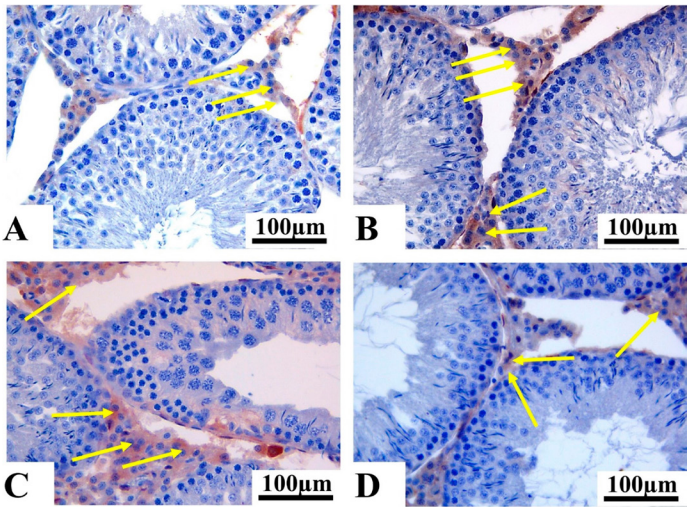


FIGURE 10. Immunoreactivity of TNF–α in testicular tissue (yellow arrow). A: Control, B: Torsion,C: Torsion+detorsion, D: Torsion+detorsion+cortexin, Immunohistochemical staining, Mayers Hematoxylin, AEC chromogen scala bar: 100 µm

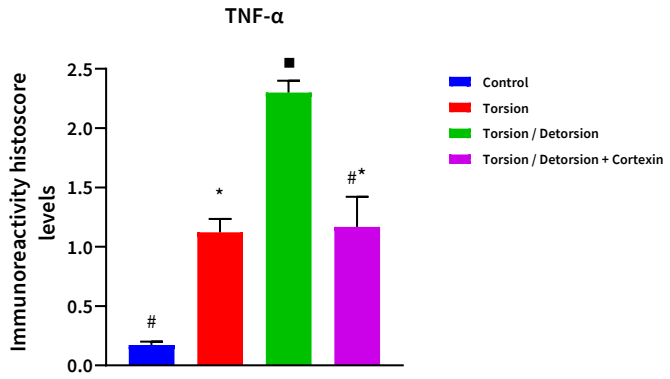


FIGURE11. TNF–α histoscore results in rat testicular tissue of all groups (#; compared to the control group (*P*<0.05), * compared to the torsion group (*P*<0.05), ▪ compared to the torsion/detorsion group (*P*<0.05)

group. However, these reductions were not statistically significant compared to the torsion group. While cortexin demonstrated potential anti–apoptotic and anti–inflammatory properties, further research is needed to fully understand its effects on Caspase–3 and TNF–α expression and to clarify its therapeutic role in mitigating I/R–induced testicular damage.

CONCLUSION

This study demonstrates that cortexin treatment significantly improves histopathological outcomes and mitigates oxidative stress in testicular tissue following ischemia–reperfusion (I/R) injury caused by testicular torsion. Cortexin reduced apoptotic cells, vacuolated epithelium, edema, vascular congestion, and apoptotic Leydig cells while restoring epithelial thickness and seminiferous tubule diameters. Although cortexin slightly decreased Caspase–3 and TNF–α expression, these reductions were not statistically significant. Additionally, cortexin increased KISS–1 and SPX expression, suggesting its potential role in protecting against testicular dysfunction. The antioxidant and anti–inflammatory properties of cortexin may contribute to its protective effects, although further research is needed to clarify its precise mechanisms and long–term benefits in testicular I/R injury.

Funding

The complete budget of the study was covered by Adıyaman University, Scientific Research Projects Coordination Unit [ADYUBAP] with project number 2023-0001.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethical Approval

The present study received ethical approval from the Adıyaman University Experimental Animals Local Ethics Committee (Approval No: 2, Protocol No: 2022/001). The experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals."

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