The renoprotective effect of Tibolone in sepsis-induced acute kidney injury

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Introduction. Sepsis-induced acute kidney injury (AKI) remains a major challenge in intensive care, contributing significantly to morbidity and mortality. Tibolone, known for its neuroprotective and hormonal properties, has not been explored for its potential in AKI management. This study investigates the protective effects of Tibolone and its underlying mechanisms involving Sirtuin-1 (SIRT1) and Yes-Associated Protein (YAP) in a rat sepsis model.
Materials and Methods. Thirty-six female Wistar albino rats underwent cecal ligation and puncture (CLP) to induce sepsis. They were randomly assigned to control, CLP+Saline, and CLP+Tibolone groups. Tibolone was administered intraperitoneally. Biomarkers, including Sirtuin (SIRT1), Yes-associated protein (YAP), Tumor necrosis factor (TNF-α), High mobility group box 1 (HMGB1), malondialdehyde (MDA), creatinine, and urea, were assessed. Histopathological examination evaluated renal damage.
Results. Tibolone administration significantly reduced plasma TNF-α, HMGB1, MDA, creatinine, and urea levels compared to the CLP+Saline group. Moreover, Tibolone elevated SIRT1 and YAP levels in kidney tissues. Histopathological examination demonstrated a significant decrease in tubular epithelial necrosis, luminal debris, dilatation, hemorrhage, and interstitial inflammation in Tibolone-treated rats.
Conclusion. This study unveils the protective role of Tibolone against sepsis-induced AKI in rats. The improvements in inflammatory and oxidative biomarkers and histological evidence suggest Tibolone's potential as a therapeutic intervention in sepsis-associated kidney injury. The upregulation of SIRT1 and YAP indicates their involvement in Tibolone's renoprotective mechanisms. Further investigations are warranted to explore Tibolone's translational potential in human sepsis-induced AKI.

Key words: Tibolone, acute renal injury, sepsis, anti-inflammatory

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INTRODUCTION

Sepsis is a pathological condition characterized by the host's dysregulated inflammatory response to an infection[1,2]. This dysregulation manifests as an imbalance between proinflammatory and anti-inflammatory factors, resulting in tissue damage and the subsequent development of multiple organ dysfunction and failure[3-5]. Sepsis continues to be the primary contributor to morbidity and mortality in intensive care units (ICUs) globally[6,7]. Sepsis-induced acute systemic inflammation triggers a series of physiological alterations that impact organ failure, particularly affecting the kidney, thereby inducing the development of AKI. Sepsis, procedure, and cardiac failure are recognized as prominent contributors to the development of AKI, with sepsis being the predominant etiological factor[8]. In certain research investigations, sepsis has been found to constitute over 30% of cases of AKI among patients. Moreover, sepsis tends to be associated with more pronounced illness and elevated mortality rates[9].

Oxidative and nitrosative stress contribute to the impairment of small blood vessels in sepsis. Additional therapies to target and resolve this problem[10] are possible. Nitro-oxidative stress contributes to the development of AKI caused by sepsis, and protein nitration is a significant mechanism involved in this process[11]. Antioxidants may be a potential therapeutic option to reduce excessive oxidative/nitrosative stress in sepsis, potentially reducing death[12].

Tibolone is an artificial steroid that exhibits estrogen-like, progestogenic, and moderate androgenic properties[13,14]. The neuroprotective effects of Tibolone have been demonstrated to occur via the activation of the Akt/GSK3ß signaling pathway. This activation leads to a reduction in Tau phosphorylation in the brain and cerebellum of ovariectomized rats. Additionally, Tibolone has been found to enhance antioxidant activity in neuronal cultures and increase the expression of the antiapoptotic protein Bcl-2. This intervention has been used to manage climacteric symptoms and osteoporosis among women in the postmenopausal stage[15,16]. Additionally, it has demonstrated advantageous antidepressant properties in women experiencing menopause[17].

Sirtuins (SIRTs) are NAD+-dependent histone deacetylases. SIRTs regulate the inflammation-related stress response in immunological and non-immune cell

types[18]. SIRT1, the sirtuin subject to the most comprehensive investigation, has been found to possess a protective influence on sepsis-induced multiple organ dysfunction associated with inflammation. The primary mechanisms of action encompass the modulation of the inflammatory response, enhancement of antioxidant capacity, and reduction of cellular apoptosis[18-20]. The lung-protective effect of SIRT has also been documented in various studies[19,20].

The Hippo signaling pathway is a pivotal regulator of organ size and the maintenance of tissue homeostasis. Hippo kinases and adaptor proteins play a crucial role in phosphorylation and subsequent inactivation of Yes-associated protein (YAP) and the transcriptional co-activator with PDZ-binding motif (TAZ), transcription co-activators that share a close relationship[21]. The Hippo/YAP pathway is important as a signaling pathway in regulating organogenesis and maintaining tissue homeostasis. YAP is a cascade effector of the Hippo circuit and is crucial in transmitting mechanical stress signals. The protein YAP plays a vital role in preserving the structural integrity of the glomerular filtration barrier. Inhibition of YAP has the potential to induce nephrotoxicity. In addition, the activation of the YAP pathway has been observed to facilitate hypertensive renal inflammation as well as fibrosis, potentially exacerbating hypertensive kidney damage. YAP has emerged as a potential therapeutic target for the mitigation and management of renal disorders[22].

This study aimed to investigate Tibolone's protective and recovery effect and related mechanisms of AKI, which the sepsis experimental model induces.

MATERIALS AND METHODS

Animals

This study utilized a sample of 36 female Wistar albino mature rats, with an average weight ranging from 200 to 250 g. The experiments conducted in this study were performed according to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health in the United States, after obtaining approval from the Animal Ethics Committee. The rats were provided with unlimited access to food. They were kept in steel cages under controlled environmental conditions, maintaining a temperature of 22 ± 2 °C and a light/dark cycle of 12 hours each.

Experimental procedures

Rats were randomly assigned into two groups, and a cecal ligation and puncture (CLP) procedure was performed on 24 rats to induce a sepsis model. Study groups were designed as follows:

- Group 1: normal control (nonoperative and orally fed, n=12);
- Group 2 (CLP+Saline, n=12): CLP and % 0.9 NaCl, intraperitoneally;
- Group 3 (CLP+Tibolone, n=12): CLP and 0.5 mg/kg/day Tibolone, intraperitoneally.

In both Group 2 and Group 3, a volume replacement treatment was administered. This treatment involved administering 30 mL/kg of 0.9% NaCl within the first hour of the surgical procedure, followed by 10 mL/kg/day of 0.9% NaCl intraperitoneally.

The treatment was divided into two equal parts at a dose of 0.25 mg/kg/day intraperitoneally for five days, each given every 12 hours during the day.

The study was concluded within five days. Seven rats died during the experiment, with six succumbing to the combined effects of cecal ligation and puncture (CLP) and saline administration. In contrast, one rat expired due to the combined effects of CLP and Tibolone administration. Upon the conclusion of the research, the animals which are alive on the fifth day were subjected to euthanasia. Subsequently, blood samples were obtained through cardiac puncture for biochemical analysis. Additionally, the kidneys were extracted to facilitate histopathological examination.

Cecal ligation and puncture (CLP) Procedure

A midline laparotomy measuring 3 cm was conducted under aseptic conditions in order to facilitate the visualization of the cecum and its adjacent intestine. The cecum was securely ligated using a 3.0 silk suture at its base, located beneath the iliocecal valve, and subsequently punctured once using a 21-gauge needle. Subsequently, gentle pressure was applied to the cecum to expel a small quantity of fecal matter from the perforation site. The cecum was reinserted into the peritoneal cavity, and the laparotomy incision was sutured closed using 4–0 polyglactin sutures. After undergoing surgical procedures, the animals were given a designated period for recuperation, after which they were subsequently placed back into their respective cages. In the control group, rats were subjected to aseptic conditions and underwent laparotomy without any ligation or puncturing of their cecum. In this experimental framework, rats were deemed septic five hours after the administration of the compound CLP (ref.[23]).

Histopathological examination of the kidney

The kidney was surgically excised and subsequently immersed in a 10% formaldehyde solution in 0.1 M phosphate-buffered saline (PBS) for three days. The kidney was divided into two parts from the long axis. Biochemical examinations were performed for the upper and histopathological examinations for the lower half. The lower half of kidney sections, which were fixed in formalin and had a thickness of 5 µm, were subjected to staining using the eosin and hematoxylin method (H&E). The sections were captured using an Olympus C-5050 digital camera affixed to an Olympus BX51 microscope.

The morphological evaluation was conducted using a computerized image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc. USA). Ten microscopic fields per section were examined at a magnification of ×20. The observer conducting the evaluation was unaware of the study group.

Pathologic scoring

Kidneys embedded in paraffin were sectioned at five μm and stained with hematoxylin/eosin by standard methods. Tubular damage was scored by calculation of the percentage of tubules in the corticomedullary junction[24] that displayed tubular epithelial necrosis, luminal necrotic debris, tubular dilatation, hemorrhage, and interstitial inflammation: score 0=0–5%, score 1=6–20%, score 2=21–40%, score 3=41–60%, score 4=61–80%, score 5=81–100%. At least ten high-power fields (magnification, ×200) per section for each sample were examined. The evaluation was conducted by assigning ratings to a predetermined scale[25].

Biochemistry evaluation of urea and creatinine

The levels of creatinine and urea concentrations were measured using a spectrophotometric method with an automated analysis system. The levels were expressed in milligrams per deciliter (mg/dL).

Kidney biochemical analysis

Following the process of decapitation, the organs were expeditiously extracted and subsequently preserved at a temperature of 20 °C until further biochemical analyses were conducted. To perform tissue analysis, the upper half of kidney samples were subjected to homogenization using a glass homogenizer. The homogenization process involved combining the kidney samples with a phosphate-buffered saline (PBS) solution, prepared at a volume five times greater than the tissue obtained. The pH of the PBS solution was maintained at 7.4. After homogenization, the resultant mixture underwent centrifugation at a centrifugal force of 5,000 times the acceleration due to gravity (5,000 g) for 15 min. The liquid portion of the sample was gathered, and the overall quantity of proteins in kidney tissue extracts was assessed utilizing Bradford's technique, employing bovine serum albumin as the reference substance. The quantification of Sirtuin-1 and Yes-associated protein (YAP) concentrations in the supernatants of kidney tissue was performed using commercially available ELISA kits designed for rats.

Determination of TNF-a, HMGB-1 levels in plasma

The plasma levels of TNF-a and HMGB-1 were measured using enzyme-linked immunosorbent assay (ELISA) kits that were commercially available and obtained from Biosciences and Abcam. The measurements were carried out according to the guidelines stipulated by the manufacturer.

Evaluation of lipid peroxidation

Lipid peroxidation was quantified in plasma samples by assessing malondialdehyde (MDA) levels as a thiobarbituric acid reactive substance (TBARS). Concisely, trichloroacetic acid and TBARS reagent were introduced to the plasma samples, subsequently combined, and subjected to incubation at 100 °C for 60 min. After cooling on ice, the samples underwent centrifugation at a speed of 3000 revolutions per minute for 20 min. Subsequently, the absorbance of the resulting supernatant was measured at a wavelength of 535 nanometers. The MDA concentrations were quantified in nanomolar units, and the assay was calibrated using tetraethoxypropane[26].

Statistical analysis

The nonparametric Mann-Whitney U test was employed to analyze all quantitative data, and the Student's t-test was employed to assess the disparities between the groups. The data are presented as mean values accompanied by the standard error of the mean (SEM). Statistical significance was attributed to *P*-values equal to or less than 0.05.

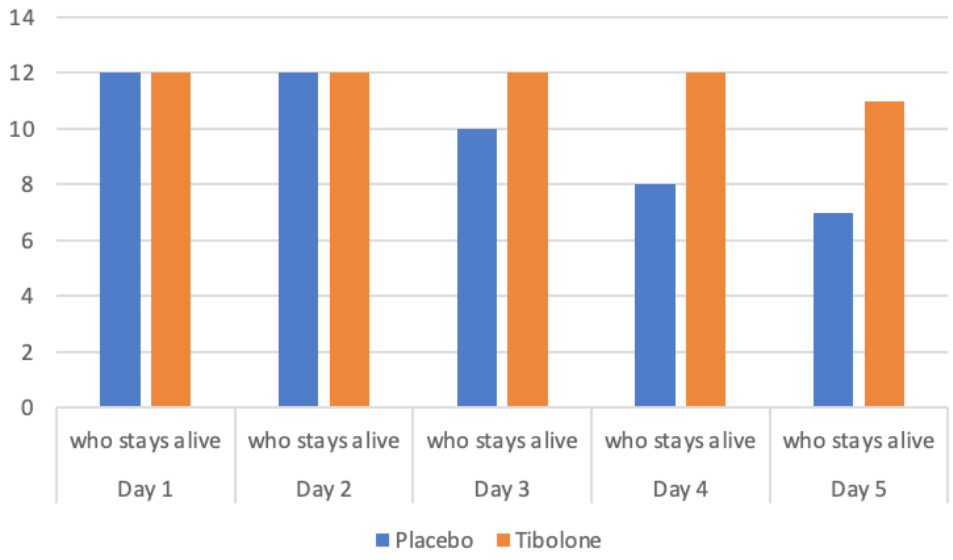

Fig. 1. Number of surviving rats in the experimental groups by day.

RESULTS

Experimental animal demographics

The study was initiated with 12 animals in each group, with daily recordings of surviving animals over the 5-day experimental period. The CLP+Saline group showed a gradual reduction in numbers (5 dead, seven survivors), whereas the CLP+Tibolone group maintained a relatively constant (1 dead, 11 survivors) count, as detailed in the following (see Fig. 1).

Biochemical Analysis

Plasma Biomarkers:

A comparison of plasma markers among the control, CLP, saline, and CLP and Tibolone groups demonstrated significant variations.

Plasma MDA (nM): Tibolone administration significantly decreased malondialdehyde levels compared to the CLP and saline group (72.4 ± 5.05# vs. 161.5 ± 11.4**, *P*<0.0001) (Table 1).

Plasma TNF-alfa (pg/mL): Tibolone reduced TNF-alfa levels (59.8 ± 4.4##) compared to the elevated levels in the CLP and saline group (103.7 ± 16.8**, *P*<0.0001) (Table 1).

Plasma HMGB1 (pg/mL): Tibolone mitigated the increase in HMGB1 levels (1.2 ± 0.3#) compared to CLP and saline (2.05 ± 0.5*, *P*<0.05) (Table 1).

Plasma BUN (mg/dl) and Creatinine (mg/dL): Tibolone administration resulted in lower BUN (28.5 ± 2.6##) and creatinine (0.32 ± 0.1#) levels compared to the CLP and saline group (*P*<0.001) (Table 1).

Kidney Biomarkers:

Kidney Sirtuin-1 (pg/mg protein): Tibolone increased Sirtuin-1 levels (3.9 ± 0.56#) compared to the reduced levels in the CLP and saline group (1.5 ± 0.2**, *P*<0.0001) (Table 1).

Kidney YAP (pg/mg protein): Tibolone elevated YAP levels (15.6 ± 1.02##) compared to the decreased levels in the CLP and saline group (8.7 ± 0.8*, *P*<0.001) (Table 1).

Histopathological Examination:

Histopathological evaluation of kidney sections further supported the protective effects of Tibolone.

Tubular Epithelial Necrosis: Tibolone significantly reduced tubular epithelial necrosis (1.6 ± 0.2##) compared to the CLP and saline group (3.3 ± 0.4*, *P*<0.001) (Table 2).

Luminal Necrotic Debris: Tibolone showed a decrease in luminal necrotic debris (1.7 ± 0.3#) compared to the CLP and saline group (2.7 ± 0.3*, *P*<0.01) (Table 2).

Tubular Dilatation: Tibolone attenuated tubular dilation (1.3 ± 0.3##) compared to the CLP and saline group (2.8 ± 0.2*, *P*<0.001) (Table 2).

Hemorrhage and Interstitial Inflammation: Tibolone exhibited protective effects against hemorrhage (0.7 ± 0.3#) and interstitial inflammation (0.4 ± 0.2#) compared to the CLP and saline group (*P*<0.01) (Table 2).

Histological Images:

Kidney histopathology images supported the histopathological findings, demonstrating decreased injury in the CLP and Tibolone groups compared to the severe alterations in the CLP and saline groups (Fig. 2).

Table 1. The result of biochemical analysis.

	Normal control	CLP and saline group	CLP and 0.5 mg/kg Tibolone group
Plasma MDA (nM)	55.6 ± 8.2	161.5 ± 11.4 **	72.4 ± 5.05 #
Plasma TNF alfa (pg/mL)	15.3 ± 1.9	103.7 ± 16.8 **	59.8 ± 4.4 ##
Plasma HMGB1 (pg/mL)	0.9 ± 0.04	2.05 ± 0.5 *	1.2 ± 0.3 #
Plasma BUN (mg/dL)	22.1 ± 0.6	41.3 ± 3.8 **	28.5 ± 2.6 ##
Plasma Creatinine (mg/dL)	0.27 ± 0.03	0.45 ± 0.02 *	0.32 ± 0.1 #
Kidney Sirtuin-1 (pg/mg protein)	4.06 ± 0.1	1.5 ± 0.2 **	3.9 ± 0.56 #
Kidney YAP (pg/mg protein)	11.2 ± 1.5	8.7 ± 0.8 *	15.6 ± 1.02 ##

Results were presented as mean ± SEM. Statistical analyses were performed by one-way ANOVA.*P<0.01, **P<0.0001 different from normal groups; #P<0.05, ##P<0.001 different from CLP and saline group.

Table 2. Semi-quantitative evaluation of tubular injury score.

	Normal control	CLP and saline group	CLP and 0.5 mg/kg/day Tibolone group
Tubular epithelial necrosis	0.1 ± 0.1	3.3 ± 0.4 *	1.6 ± 0.2 ##
Luminal necrotic debris	0.2 ± 0.1	2.7 ± 0.3 *	1.7 ± 0.3 #
Tubular dilatation	0.1 ± 0.1	2.8 ± 0.2 *	1.3 ± 0.3 ##
Hemorrhage	0.1 ± 0.1	1.5 ± 0.2 *	0.7 ± 0.3 #
Interstitial inflammation	0.1 ± 0.1	0.9 ± 0.2 *	0.4 ± 0.2 #

Results were presented as mean ± SEM. Statistical analyses were performed by one-way ANOVA and post-hoc Bonferroni test. *P<0.001 different from normal groups; #P<0.01, ##P<0.001 different from CLP and saline group.

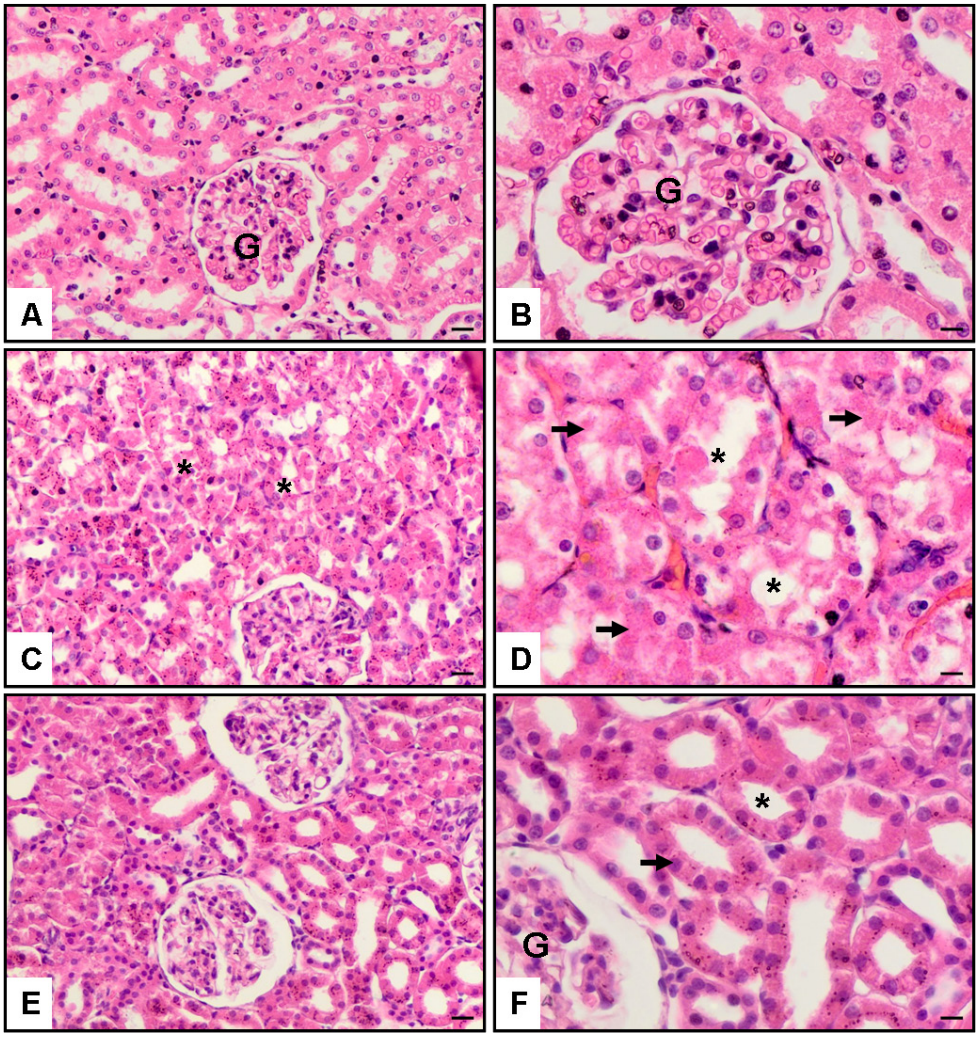

Fig. 2. Kidney histolopathology x10 (A,C,E) and x 20 (B,D,F) magnification, hematoxylin and eosin (H&E) stain.
A,B: Normal group kidney, renal tubules; G, Glomerulus; C,D: CLP and saline groups was showed severe histopathologic alteration related to tubular injury that dilatation(*) and necrosis (arrow); E,F: CLP and 0.5 mg/kg Tibolone groups was showed decreased injury.

DISCUSSION

Sepsis is a disease state that has been continuously studied in terms of lung and kidney damage, yet no common treatment protocol has been established. This study not only provides strong evidence for the anabolic, anti-inflammatory, and antioxidant effects of Tibolone but also explains how Tibolone affects the context of YAP and Sirtuin-1 pathways.

In a multicenter study involving a total of 29,269 critically ill patients, it was observed that septic shock (47.5%) was identified as the primary contributing factor to AKI (ref.[27]). Furthermore, substantial evidence suggests that the mortality rate among patients diagnosed with AKI, particularly those necessitating dialysis interventions, can exceed 70% (ref.[28,29]). Furthermore, Medieros et al. provide a description indicating that Tibolone and 17-estradiol exhibit effective augmentation of plasma atrial natriuretic peptide (ANP) levels and ANP messenger RNA (mRNA) levels in the left atrium[30]. However, these treatments do not restore renal levels of natriuretic peptide receptor-A (NPR-A) in female rats with estrogen deficiency. As we saw, estrogen has strong anti-inflammatory properties. This study proves the acute renal injury and repairs with Tibolone by creatine and BUN levels. As far as the existing body of literature is concerned, there is a lack of studies examining the utilization of Tibolone in cases of acute renal injury or failure. This study is the first investigation showing the protective effect of Tibolone in ARI.

HMGB1, a potent proinflammatory cytokine, is recognized as a late-released proinflammatory mediator in the inflammatory cascade[31]. The HMGB1 protein is present in the nuclei of all cell types. Many inquiries have examined the potential advantages of utilizing antibodies or selective antagonists to target HMGB1 in various preclinical studies of inflammation-related diseases, including sepsis and lethal endotoxemia. These investigations have demonstrated promising protective effects[31-33]. Karaali et al., in a feces-induced peritonitis sepsis model in which they searched about the effect of beta blockers in lung injury, found, similar to our study, the augmented HMGB1 levels

in the sepsis group[31]. In this study, early inflammatory markers (TNF-A, MDA) and late inflammatory markers (HMGB1) behaved like any inflammatory and oxidative processes that cause inflammatory and oxidative processes. They went up and down when Tibolone was given to suppress them.

Zhang et al.[22] demonstrated a reduction in the expression of SIRT1 in the cell model of AKI induced by lipopolysaccharide (LPS). The study by Qiufang Gao observed the successful overexpression of SIRT1 in the HK-2 cell line, which led to an increase in cellular proliferation and a decrease in the apoptosis rate. In a study conducted by Fan et al.[34], it was observed that the lack of SIRT1 led to a heightened occurrence of cellular apoptosis in cases of AKI induced by ischemia/reperfusion. Furthermore, as an illustrative instance, in a murine model of sepsis involving ligation and puncture, SIRT1 exhibits a mitigating effect on sepsis-induced lung inflammatory injury by suppressing the activation pathway of the inflammasome and reducing the synthesis of proinflammatory mediators[19]. A recent investigation has demonstrated that the excessive expression of SIRT1 promotes the growth of renal epithelial cells while inhibiting their programmed cell death, thereby improving the condition of AKI induced by lipopolysaccharide in sepsis[20]. While there is growing recognition of the mechanisms by which SIRT1 is involved in sepsis, the clinical implications of SIRT1 in sepsis patients have yet to be fully understood. The study conducted by Hasegawa and colleagues provides evidence supporting the protective role of SIRT1 in renal tubules against diabetic albuminuria. This protection is achieved by maintaining nicotinamide mononucleotide (NMN) concentrations around glomeruli and modulating podocyte function[35,36]. The objective of this current study was also to examine the potential of SIRT1 as a biomarker for managing disease and predicting prognosis in individuals with sepsis, as in the study of Cheng et al.[37]. Our findings indicate that the expression of SIRT1 was downregulated in the septic group, and the anti-inflammatory effect of Tibolone was augmented.

YAP inhibition triggers the AKI in septic patients[38]. For example, the YAP signaling pathway facilitates the apoptosis of human pulmonary microvascular endothelial cells induced by lipopolysaccharide (LPS). Consequently, the inhibition of YAP could serve as a promising therapeutic approach for managing lung injury in septic conditions[38]. On the other hand, YAP plays a crucial role in maintaining the integrity of the glomerular filtration barrier, and its inhibition may cause proteinuric kidney disease and potentially cause nephrotoxicity[39]. A study conducted by Chen et al. observed that YAP activation, a critical factor in epithelial cell regeneration during kidney recovery from AKI, is essential for kidney recovery from AKI (ref.[40]). On the other hand, after YAP activation, repair can be maladaptive and provoke inflammation[41]. While inhibition of the HIPPO/YAP pathway indicates acute kidney damage, the increase in YAP activated after Tibolone use indicates cessation of kidney damage and even regeneration, as in our study.

Histopathologically, as in Aslan et al.[42] study, we observed the tubular and glomerular degradation and recovery in the sepsis group after 5 mg/kg Tibolone admission. After Tibolone implementation, we can also confirm these changes and improvements in tubular epithelial necrosis, Luminous necrotic debris, tubular dilatation, hemorrhage, and interstitial inflammation scores. Moreover, during the five days of the study, we saw five dead rats in the CLP+Saline group and just one dead in the Tibolone group. Here, we can comment that Tibolone improves renal injury and sepsis and makes rats survive through its anabolic, antioxidant, and anti-inflammatory properties.

In previous studies, Tibolone has been studied in models of nerve injury, Alzheimer's disease, depression, and cardiovascular diseases[43-46]. For instance, Hidalgo-Vanessa et al. found that administering Tibolone has been observed to mitigate oxidative damage and inflammation in microglia stimulated with palmitic acid[47]. We can observe the steroid effect of Tibolone in this type of research. In contrast to studies on the evidence of its neuroprotective properties, the present study is the first to present evidence of sepsis-induced kidney injury. We think that Tibolone is a drug that should be recommended for sepsis, especially in the early treatment process.

Limitations

As this study is experimental in animal models, there is no evidence of human uses. It is important to point out the differences between the pathophysiology of sepsis in rats and humans. As there is no other study with intraperitoneal use of Tibolone, Tibolone administration at doses higher or lower than 0.5 mg/kg should be repeated, and a dose-response curve should be established.

CONCLUSİON

In this study, we prove the protective and healing effect of Tibolone by using anti-inflammatory and antioxidative biomarkers to demonstrate histological changes. Further studies will provide more significant and precise information on the regulatory effects of Tibolone.

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