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The impact of the sepsis on female urogenital system: the role of pregabalin

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Abstract

Purpose The aim of the study was to investigate the oxidative damage and inflammatory effects of sepsis on the urogenital system in the Lipopolysaccharide (LPS)-induced sepsis model and ameliorating role of Pregabalin (PGB).

Methods Twenty-four female Wistar Albino rats (12 months old) were divided into 3 groups as follows: Sepsis group (Group S) (5 mg/kg LPS, i.p, single dose); Sepsis+PGB group (Group SP) (5 mg/kg LPS, i.p, single dose and 30 mg/kg PGB); Control group (Group C) (0.1 ml/oral and i.p. saline, single dose), 6 h after LPS administration, the animals were killed. Subsequently, analyses of urogenital tissue oxidant/antioxidant status, histopathological and immunohistochemical analyses were performed.

Results Total oxidative status (TOS) and oxidative stress index (OSI) values in the urogenital tissues were increased in Group S (Total anti-oxidative status (TAS) decreased) compared to the Control group ($p < 0.05$). PGB improved these values ($p < 0.05$). The immunohistochemical markers [Caspase-3, granulocyte colony-stimulating factor (G-CSF), interleukin-6 (IL-6), Serum Amyloid A (SAA) and inducible nitric oxide synthase (iNOS)] were significantly increased in Group S except for bladder ($p < 0.001$). Statistically significant immunohistochemical positiveness was found only for IL-6 in urinary bladder, though all the others values were negative. With the administration of PGB (Group SP), the expressions of these immunoreactions were markedly decreased ($p < 0.001$).

Conclusions These findings demonstrated that sepsis caused oxidative stress and inflammation in the urogenital tissues. We have revealed that PGB ameliorated tissue damage caused by sepsis.

Keywords Urogenital system · Lps-induced rat sepsis · Pregabalin

Introduction

Sepsis is defined as a life-threatening multiple organ dysfunction caused by an uncontrolled host response to infections that is mediated by the secretion of proinflammatory mediators [1]. Sepsis is a serious public health problem which is not sufficiently known, quite costly to diagnose, with a high mortality and morbidity rates. The mortality rate for sepsis in hospitalized patients is 17–26% depending on the severity of disease [2].

Sepsis-induced multiple organ failure is seen in more than 70% of sepsis patients and it is a leading cause of mortality in sepsis [3]. The most important pathophysiological causes of mortality in sepsis are hypoperfusion, coagulopathy, metabolic acidosis, septic shock and, as previously mentioned, multiple organ failure [4].

The most important hallmark of the sepsis is the systemic inflammation followed by the excessive production of the proinflammatory mediators which results in oxidative damage, mitochondrial dysfunction, cellular and multiple organ damages [5]. Systemic inflammation can be generated in various tissues such as endothelial, renal tubular epithelial, brain, hepatic and lung tissues via experimental LPS-induced sepsis model [6]. Still, the overall pathophysiology of sepsis is not yet completely understood [7].

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Serum amyloid A is an acute-phase indicator which is secreted by the liver in response to any inflammation such as infection, trauma or sepsis [8]. IL-6 is an important pro-inflammatory cytokine which is produced during sepsis [5, 9]. Nitrosative stress (iNOS expression, NO content, plasma total nitrite content, etc.) is another important marker by which sepsis severity and consequently resulting organ toxicity were evaluated [10]. G-CSF is another marker examined in our study because of the fact that production and expression of G-CSF increases during sepsis due to the sepsis-related immune system disturbances [11].

Pregabalin (PGB) is a substance with anti-apoptotic, anti-inflammatory, and anti-oxidative properties PGB have multifunctional effects in the sepsis. Apoptosis, inflammation, and oxidative stress inhibit by PGB in sepsis. These effects have been demonstrated in the literature with analyses of specific markers in various pathways to elucidate the deleterious effects of sepsis [12–18]. The reason for preferring PGB in our study was due to the properties mentioned above. PGB was first used in medicine as an anticonvulsant drug for epilepsy treatment. Nowadays, PGB is widely used in medicine. Its major characteristics are: sedative [19], anesthetic adjuvant, treatment of general anxiety disorders [20], analgesic (fibromyalgia, neuropathic pain, acute and chronic sciatica pain) [21], treatment of erectile dysfunction, anticonvulsant [22], anti-nociceptive effect, non-hormonal treatments of vasomotor symptoms in postmenopausal term, cancer patients [23], vulvodynia [24], urological chronic pelvic pain syndrome, and acute pain after any surgical procedure [25].

In the present study, we evaluated the histopathologic and immunochemical changes, inflammatory responses, and oxidative stress status (Caspase-3, G-CSF, IL-6, SAA, iNOS immunoreactivity, TAS, TOS, OSI levels) which develop in response to LPS-induced sepsis and the effects of PGB on urogenital area.

As we know, there was no other paper in the medical literature studying sepsis and PGB's effects on female urogenital tissues and reproductive tract until today.

Methods

Animals

Twenty-four female Wistar Albino rats (12 months old) were obtained from the Experimental Animal Production and the Experimental Research Laboratory of Mehmet Akif Ersoy University (Burdur, Turkey). The procedures performed on rats were reviewed and approved by the Animal Experiments Local Ethics Committee of Mehmet Akif Ersoy University (Ethic No: 309, August 2017/02).

The animals were kept under standard laboratory conditions (temperature, 21–23 °C, humidity, 55–60% and 12-h light/dark cycle). All the groups received standard commercial chow diet (Korkuteli Yem®) and tap water. Other environmental factors were kept the same in all the groups. Before starting the experiment, the animals had been monitored for 7 days for orientation.

Chemicals

Lipopolysaccharide was obtained from the Sigma (500 mg flacon, 048K4126, Sigma Aldrich, St. Louis, MO, USA) and applied intraperitoneally as a single dose. A commercial form of PGB (300 mg tablets PGB-Lyrica, Pfizer, Turkey) was used for the treatment. All chemicals were of analytical grade, obtained from Sigma-Aldrich Chemical Inc. (Sigma Aldrich, St. Louis, MO, USA), and all organic solvents were purchased from Merck Chemical Inc. (Istanbul, Turkey). All solutions, except phosphate buffers, were prepared daily and stored at +4 °C. The reagents were allowed to equilibrate at room temperature for at least 30-min before used for analysis. The phosphate buffers were stable and stored at +4 °C for 1 month.

Experimental design

Twenty-four female Wistar Albino rats (12 months) were randomly divided into three groups as an equally: Sepsis group (Group S, exposure to Lipopolysaccharide), Sepsis + Pregabalin group (Group SP, exposure to Lipopolysaccharide + PGB) and Control group (Group C). The control group was fed with standard commercial chow diet and tap water. Saline in a volume equivalent to PGB and LPS dosages used in the study was administered orally to the control group. A single dose of LPS (5 mg/kg, i.p) and saline in a volume equivalent to PGB dose given orally were applied to the rats in S group. The rats in SP group, received a single dose of LPS (5 mg/kg LPS, ip) and PGB (30 mg/kg orally) [26]. PGB dissolved in normal saline [27] was administered 1 h before LPS administration.

Animals were killed 6 h after LPS administration by exsanguination, following the standardized ethical procedures. Blood samples were collected from each animal for biochemical analyses and comet assay. Plasma samples were obtained by centrifugation at 4000 rpm for 10 min and stored at − 80 °C until analyzed. Urogenital tissues (ovarium, tuba uterina, uterus, cervix, vagina, urinary bladder) were rapidly dissected. Ovarium, tuba uterina and uterus tissues were precisely divided into two parts. One part of these tissues and another part of the whole tissue were kept in 10% formalin solution for histological examination. The other parts were homogenized and stored at − 80 °C until used for oxidant/antioxidant analyses.

Biochemical analyses

The Ovarium, tuba uterina and uterus tissues extracted from the killed animals were first placed in phosphate buffer (pH 7.4).

Briefly, the tissues were disrupted with a homogenizer (IKA Ultra-Turrax T25 Basic; Labortechnic, Staufen, Germany) and a sonicator (UW-2070 Bandelin Electronic, Germany). Later, the tissue samples were centrifuged at 10,000*g* for 10 min at + 4 °C. The colorimetric and automated methods developed by Erel for the evaluation of total anti-oxidative status (TAS) and total oxidative status (TOS) in tissue samples were used [28]. The change in absorbance of samples was measured at 660 nm using a spectrophotometer (Shimadzu UV1601 spectrophotometer, Japan) and the results were expressed as mmol Trolox Eq/mg protein. Oxidative stress index (OSI) was defined by the formula OSI (arbitrary unit) = [(TOS, mmol/L)/(TAS, mmol Trolox equivalent/L)/100].

Histopathological examinations

During necropsy, the genital system (ovarium, tuba uterina, uterus, cervix and vagina) and urinary bladder tissue samples were collected and fixed in 10% buffered formalin. After the routine processing by an automatic tissue processor equipment (Leica® ASP300S, Wetzlar, Germany), tissues were embedded in paraffin and sectioned at 5-µm thickness by a Leica RM2155 rotary microtome (Leica Microsystems®, Wetzlar, Germany). Tissue sections were stained with hematoxylin–eosin (H&E) and examined microscopically.

Immunohistochemical examinations

The selected samples were immunostained with caspase-3 [Anti-Caspase-3 antibody (ab4051)], granulocyte colony-stimulating factor [Anti-G-CSF antibody (ab9691)], interleukin-6 [Anti-IL6 antibody (ab9324)], iNOS [Anti-iNOS antibody (ab15323)], and Serum Amyloid A [Anti-Serum Amyloid A antibody [mc1] (ab655)] by streptavidin biotin technique. All primary and secondary antibodies were purchased from Abcam (Cambridge, UK). The sections were incubated with the primary antibodies for a period of 60 min, and immunohistochemistry was carried out using biotinylated secondary antibody and streptavidin–alkaline phosphatase conjugate. EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) was used as a secondary antibody. The antigens were demonstrated using diaminobenzidine (DAB) as the chromogen. For negative controls the primary antiserum step was omitted. All the examinations were performed in a blinded manner. The percentage of positively immunostained cells for each marker was counted in 10 different fields for every section at X40 objective magnification in all groups. Results obtained from the image analyzer were subjected to statistical analysis. Morphometric analyses were performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical analysis

Variables were presented as frequencies, percentages, mean ± standard deviations, median or min–max. Kolmogorov–Smirnov and Shapiro–Wilk tests were used to test for a normal distribution of continuous variables, and Levene test was used for homogeneity of variance. Data characterized by a normal distribution were expressed as mean ± standard deviation. Parameters without such distribution were expressed as median with range. The groups were compared using non-parametric Kruskal–Wallis test and Mann–Whitney *U* test. Biochemical parameters were shown to fit with the normal distribution, and ANOVA and post hoc Bonferroni, LSD tests were used to compare the groups. The immunopositive cells were used for immunohistochemical analysis. Calculations were made using the SPSS 22.0 program pack (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

Results

Ovarian tissue

There were significant changes in TAS, TOS and OSI levels ($p < 0.05$) in ovarian tissue among the groups. In ovarian tissues, a statistically significant increase at TOS levels ($p = 0.039$) and OSI index ($p = 0.006$) was observed in S group compared to C group. TAS levels were increased in SP group and found statistically different ($p = 0.001$) compared to S group. However, a significant decrease in TOS levels ($p = 0.011$) and OSI index ($p = 0.001$) was observed in SP group) compared to S group (Table 1).

The histopathological examination of ovarium revealed a normal ovarial tissue architecture in C group (Fig. 1). But severe hyperemia, hemorrhages or neutrophil leukocyte infiltrations were seen in ovarian tissue in S group. PGB treatment ameliorated these pathological findings in ovarian tissue (Fig. 1).

The immunohistochemical examination revealed that LPS caused sepsis and increased Caspase-3, G-CSF, IL-6, SAA and iNOS expressions in all urogenital organs, PGB treatment ameliorated the immunohistochemical expressions of the inflammatory markers (Fig. 1). The statistical analysis of the immunohistochemically positive cells is shown in Table 4.

Table 1 Oxidative stress markers of ovarium tissues

Groups	TAS (mmolTroloxequivalents/L)		TOS (µmol H2O2 Equiv./L)		OSI	
	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value
Control	0.40 ± 0.07		20.37 ± 5.56		33.31 ± 19.29	
S	0.32 ± 0.06	a: 0.001	30.57 ± 4.45[a]	a: 0.039	63.35 ± 11.18[a]	a: 0.006
SP	0.66 ± 0.15ab	b: 0.001	17.70 ± 4.75[b]	b: 0.001	19.55 ± 14.31[b]	b: 0.001

Values are presented as means ± SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA

S sepsis, *P* pregabalin, *TAS* total antioxidant levels, *TOS* total oxidant levels, *OSI* oxidative stress index

a: $p < 0.05$ versus control group, b: $p < 0.05$ versus Sepsis group

Fallopian tubes

There were significant changes in TOS levels ($p < 0.05$), but not in TAS and OSI levels ($p > 0.05$) in fallopian tubes among the groups. A significant increase was observed in TOS levels in fallopian tube tissues ($p = 0.035$) in S group compared to C group. A significant decrease was observed in TOS levels in fallopian tube tissues ($p = 0.014$) in SP group compared to S group (Table 2).

Histopathological examination of the fallopian tube revealed normal fallopian tube tissue in the control group. In S group, severe hyperemia and occasional hemorrhages or neutrophil leukocyte infiltrations were found in fallopian tube tissue. PGB treatment ameliorated these pathological findings (Fig. 2).

Immunohistochemical examination revealed that LPS caused sepsis and increased Caspase-3, G-CSF, IL-6, SAA and iNOS expressions in all fallopian tubes. PGB treatment decreased the immunohistochemical expressions of the inflammatory markers (Fig. 2). The results of statistical analysis of the immunohistochemically positive cells are shown in Table 4.

Endometrial tissue

There were significant changes in TAS, TOS, and OSI levels ($p < 0.05$) in the endometrial tissue among the groups. In endometrial tissue, a statistically significant increase in TOS levels ($p = 0.001$) and OSI index ($p = 0.001$) was observed in S group compared to C group. A statistically significant decrease in TOS levels ($p = 0.012$) was observed in SP group compared to C group. However, a significant increase in TAS levels ($p = 0.018$) was observed in S group compared to C group, and a significant decrease in TAS levels ($p = 0.025$) was observed in SP group compared to S group (Table 3).

Histopathological examination of the endometrium revealed normal endometrial tissue architecture in the control group. Severe hyperemia and occasional hemorrhages or neutrophil leukocyte infiltrations were found in the endometrial tissue of S group. PGB treatment ameliorated all these pathological findings (Fig. 3).

Immunohistochemical examination revealed that LPS caused sepsis and increased Caspase-3, G-CSF, IL-6, SAA and iNOS expressions in endometrial tissue. PGB treatment decreased the immunohistochemical expression of the inflammatory markers (Figs. 1, 2, 3, 4, 5, 6). The results of statistical analysis of the immunohistochemically positive cells are shown in Table 4.

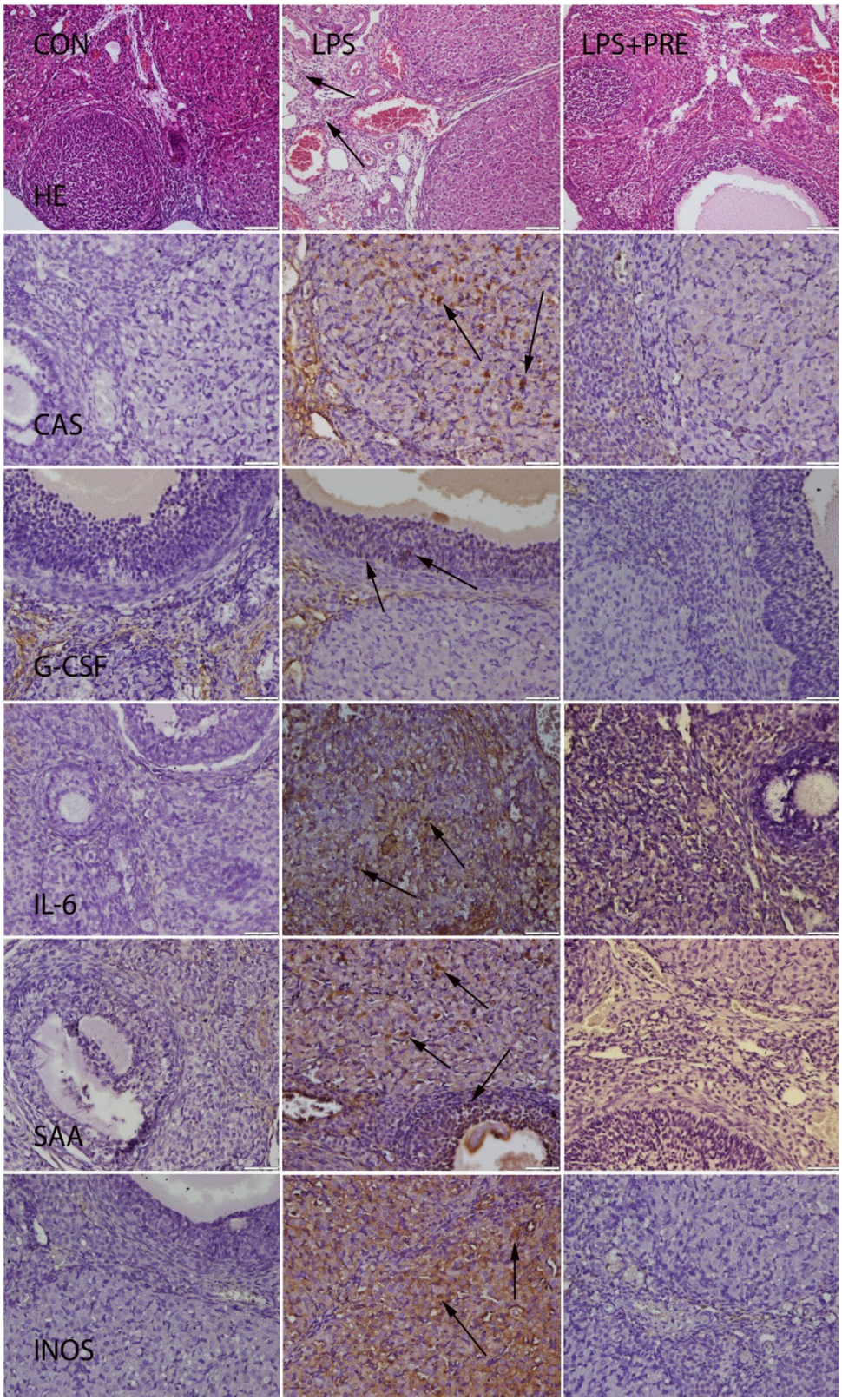

Fig. 1 First row: ovarium histopathology: normal histology in C group; severe hyperemia and slight neutrophil infiltration (arrows) in stroma of the ovarium in S group, almost normal histology except slight hyperemia in SP group, H&E, bars = 100 µ. Second row; Cas-3, third row: G-CSF, fourth row: IL-6, fifth row: SAA and sixth row: iNOS immunoreaction. No expression in C group; numerous immunopositive cells (arrows) in corpus luteum in S group, Negative reaction in SP group, streptavidin biotin peroxidase method, bars = 50 µ

Table 2 Oxidative stress markers of fallopian tubes

Groups	TAS (mmolTroloxequivalents/L)		TOS (μmol H2O2 Equiv./L)		OSI	
	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value
Control	0.97 ± 0.07		11.28 ± 3.50	NS	12.32 ± 4.76	
S	0.94 ± 0.15	NS	17.77 ± 3.44[a]	a: 0.035	17.50 ± 6.85	NS
SP	1.05 ± 0.19	NS	10.35 ± 6.10[b]	b: 0.014	11.08 ± 5.02	NS

Values are presented as means ± SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA

S sepsis, *P* pregabalin, *TAS* total antioxidant levels, *TOS* total oxidant levels, *OSI* oxidative stress index, *NS* not significant

a: $p < 0.05$ versus control group, b: $p < 0.05$ versus Sepsis group

Uterine cervical tissue

Histopathological examination of the uterine cervix revealed normal cervical tissue architecture in the control group. Severe hyperemia and occasional hemorrhages or neutrophil leukocyte infiltrations were found in the cervical tissue of S group. PGB treatment ameliorated these pathological findings (Fig. 4).

Immunohistochemical examination revealed that LPS caused sepsis and increased Caspase-3, G-CSF, IL-6, SAA and iNOS expressions in cervical tissue. PGB treatment decreased the immunohistochemical expression of the inflammatory markers (Fig. 4). The results of statistical analysis of the immunohistochemically positive cells are shown in Table 4.

Vaginal tissue

Histopathological examination of the vagina revealed normal vaginal tissue architecture in the control group. Severe hyperemia and occasional hemorrhages or neutrophil leukocyte infiltrations were found in vaginal tissue of S group. PGB treatment ameliorated the pathological findings (Fig. 5).

Immunohistochemical examination revealed that LPS caused sepsis and increased Caspase-3, G-CSF, IL-6, SAA and iNOS expressions in vaginal tissue. PGB treatment decreased the immunohistochemical expression of the inflammatory markers (Fig. 5). The results of statistical analysis of the immunohistochemically positive cells are shown in Table 4.

Urinary bladder tissue

Histopathological examination of the urinary bladder revealed normal urinary bladder tissue architecture in the control group. In S group, severe hyperemia and occasional hemorrhages or neutrophil leukocyte infiltrations were seen in urinary bladder tissue. PGB treatment ameliorated the pathological findings. Interestingly, urinary bladder was the least affected organ compared to other genital system organs in this study (Fig. 6).

Immunohistochemical examination revealed that LPS caused sepsis, but no increase was observed in Caspase-3, G-CSF, IL-6, SAA and iNOS expressions in urinary bladder tissue in S group. PGB treatment did not cause a statistically significant decrease in the immunohistochemical expression of the inflammatory markers (Fig. 6).

Immunohistochemical expressions of all markers were not significantly changed in urinary bladder except for IL-6. The results of statistical analysis of the immunohistochemically positive cells are shown in Table 4.

Discussion

Medical literature regarding sepsis is generally focused on its life-threatening part. But there are no sufficient data on the effects of sepsis on urogynecological area and reproductive function in sepsis survivors. As far as we know, our study is the first study which totally and comprehensively evaluates the effects of sepsis on the urogenital system with the help of the analyses of oxidative stress, apoptosis pathways, diffuse inflammatory responses, and immunohistopathological staining.

Many protective and beneficial effects of PGB on urogynecological tissues were detected by means of the current study. Hence, PGB may also provide a novel approach to antisepsis therapy due to the favorable features such as cost-effectivity, a favorable linear pharmacokinetic profile, absence of interaction with foods and plasma proteins, good tolerance, as well as unchanged excretion by kidneys. In this study, we showed that the harmful effects of sepsis mentioned above were significantly decreased by PGB via multiple pathways.

Aslankoc et al. in the study conducted on septic female rats revealed that the sepsis group had higher values of TOS, and OSI in brain tissues compared to the control

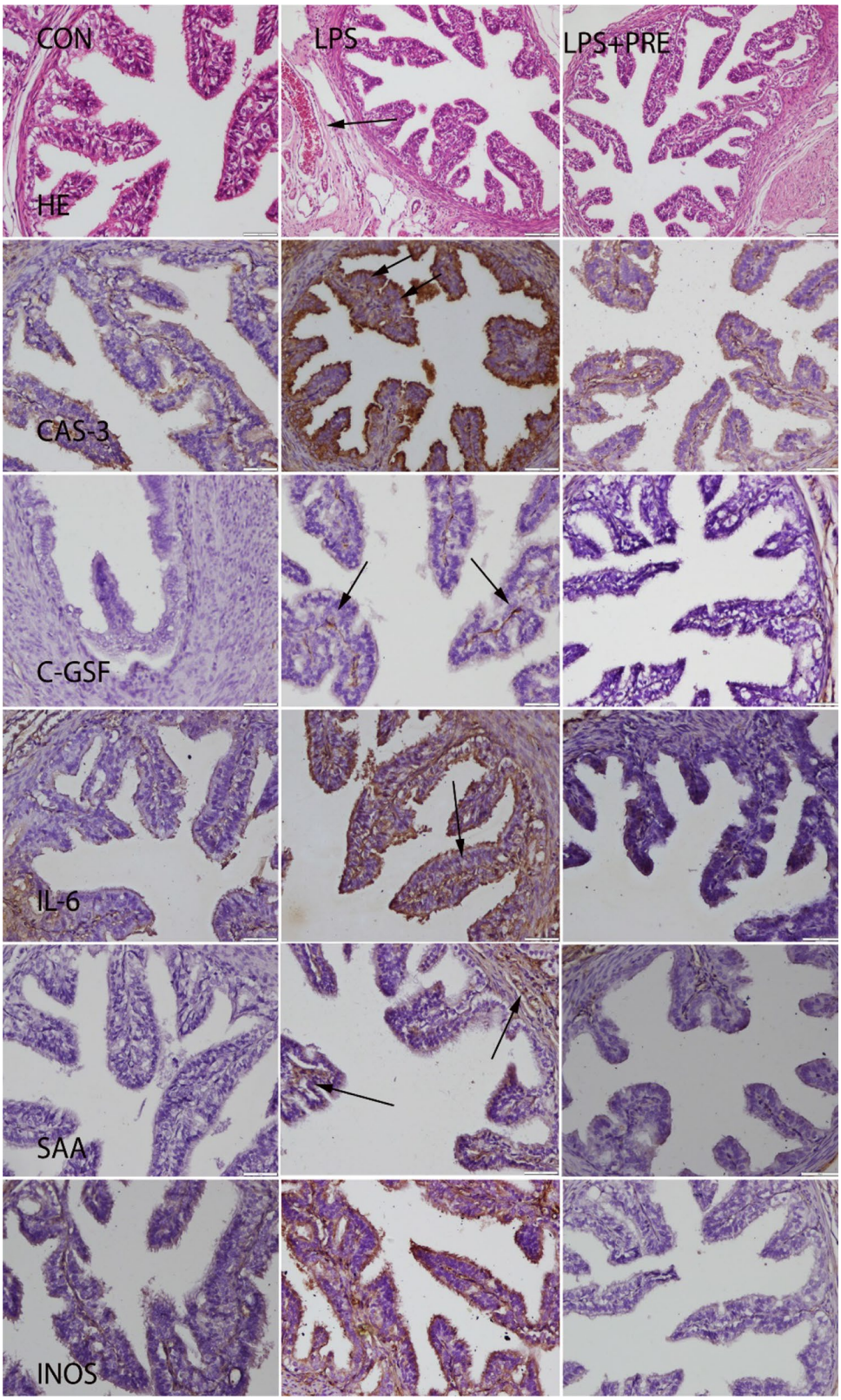

Fig. 2 Tubal histopathology: normal tubal architecture in C group; severe hyperemia (arrow) in submucosa, no pathological findings in SP group, H&E. Tubal immunohistochemistry: second row; Cas-3, third row: G-CSF, fourth row: IL-6, fifth row: SAA and sixth row: iNOS immunoreaction of the tuba uterina. Slight expression in C group; increased immunopositive reaction in the tubal cells (arrows) in S group, no very slight reaction in SP group, streptavidin biotin peroxidase method, bars = 50 µ

group. Some of these results (TOS and OSI) were similar to our results obtained from urogenital tissues (ovarium, endometrium). Similarly to our study, Aslankoc et al. found lower TOS and OSI levels in brain tissues in PGB groups in their study compared to the sepsis group [29]. These data showed some discrepancy from our results because

Table 3 Oxidative stress markers of endometrium

Groups	TAS (mmolTroloxequivalents/L)		TOS (μmol H2O2 Equiv./L)		OSI	
	Mean ± SD	*p* value	Mean ± SD	*p* value	Mean ± SD	*p* value
Control	1.01 ± 0.16		8.04 ± 1.91		9.76 ± 2.56	
S	0.64 ± 0.16[a]	a: 0.018	13.66 ± 2.06[a]	a: 0.001	20.34 ± 4.35[a]	a: 0.001
SP	1.36 ± 0.34[ab]	a: 0.025 b = 0.001	9.90 ± 2.16[a,b]	a: 0.012 b: 0.042	10.75 ± 02.23[b]	b: 0.001

Values are presented as means ± SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA

S sepsis, *P* pregabalin, *TAS* total antioxidant levels, *TOS* total oxidant levels, *OSI* oxidative stress index, *NS* not significant

a: $p < 0.05$ versus control group, b: $p < 0.05$ versus Sepsis group

the levels of TAS in our study were found lower in sepsis tissues except for fallopian tubes. However, TAS decreases in them were statistically insignificant. In this context, we first evaluated oxidative stress status (TAS, TOS, OSI) in ovarium, uterus and fallopian tubes in sepsis rats. TOS levels were significantly decreased in SP group compared to S group. OSI levels were significantly decreased with PGB in ovarium and uterus (except for tuba uterina). OSI levels were significantly decreased with PGB in ovarium and uterus (except tuba uterina). However, TAS levels were significantly higher in SP group (except for tuba uterina). These results demonstrated that oxidative stress was reduced by PGB in all three tissues. The histopathological examinations of the urogenital system revealed normal tissue architecture in the control group. In sepsis group, we revealed several pathological signs such as severe hyperemia, occasional hemorrhages or neutrophil leukocyte infiltrations (Figs. 1, 2, 3, 4, 5, 6). PGB treatment ameliorated the pathological findings (except for urinary bladder). Interestingly, urinary bladder was the least affected organ compared to other genital system organs in the current study. We have detected that statistically positive IL-6 immunochemistry staining in all tissues was decreased in SP group compared to S group (Table 4). Riedemann NC et al. in the experimental study demonstrated that IL-6 blockade in sepsis had a protective role in which survival was significantly improved with the administration of anti-IL-6 treatment [30]. In an another study, Ding et al. demonstrated that IL-6 played an important role in the damage of cardiac, liver and renal functions in the sepsis rats through SIRT 1 [5] since mRNA and protein of IL-6 levels were increased in the sepsis rats. On the other hand, Rosengarten et al. revealed that IL-6 values endotoxic rat shock model were significantly higher in sepsis group compared to the control group [31]. All these data were similar to our data. These results suggested that the addition of PGB to standard sepsis treatment could improve reproductive function among female sepsis survivors. Nowadays, scientists have been continuously working to develop such adjuvant therapies. For this reason, we aimed to explore the potential ameliorative effects of PGB on urogenital area due to its protective, anti-inflammatory, anti-apoptotic, and antioxidant properties.

Our study showed that Caspase-3 immunohistochemical staining was significantly high in LPS-induced sepsis in all urogenital tissues (except for bladder tissue) which was ameliorated with PGB administration (especially in vagina, ovarium and tuba uterina, nearly similar to the control group). Increased immunohistochemical positiveness of Caspase-3 indicates that apoptosis and inflammation in tissues secondary to septic damage.

Similarly to our study, Lorente et al. demonstrated that Caspase-3 levels were associated with sepsis (especially, sepsis severity), degree of apoptosis, and early sepsis mortality [32].

In another study, Metukuri MR et al. demonstrated that the overexpression of Caspase-3 shows the involvement of stress response proteins and mitochondrial dysfunction in LPS-induced germ cell death and apoptosis in male rats [33]. According to our findings, PGB administration in addition to standard sepsis therapy may prevent the damage caused by sepsis with the help of its anti-apoptotic, antioxidant and anti-inflammatory effects.

We also showed that all urogynecological tissues except for urinary bladder were damaged by sepsis and the results were ameliorated by PGB reaching the same results as in the control group in terms of SAA and IL-6 levels. SAA is an immune inflammatory biomarker which is secreted by the liver in response to the inflammation such as infection, trauma and sepsis [8]. SAA analysis is very important for diagnosing adult and neonatal sepsis [34]. Fu et al. demonstrated that procalcitonin (PCT), C-reactive protein (CRP), IL-6 and SAA were increased in sepsis group compared to non-sepsis group [35].

Inducible nitric oxide synthase is another important inflammatory mediator of sepsis. In our study, we also detected statistically significant positive immunoreaction

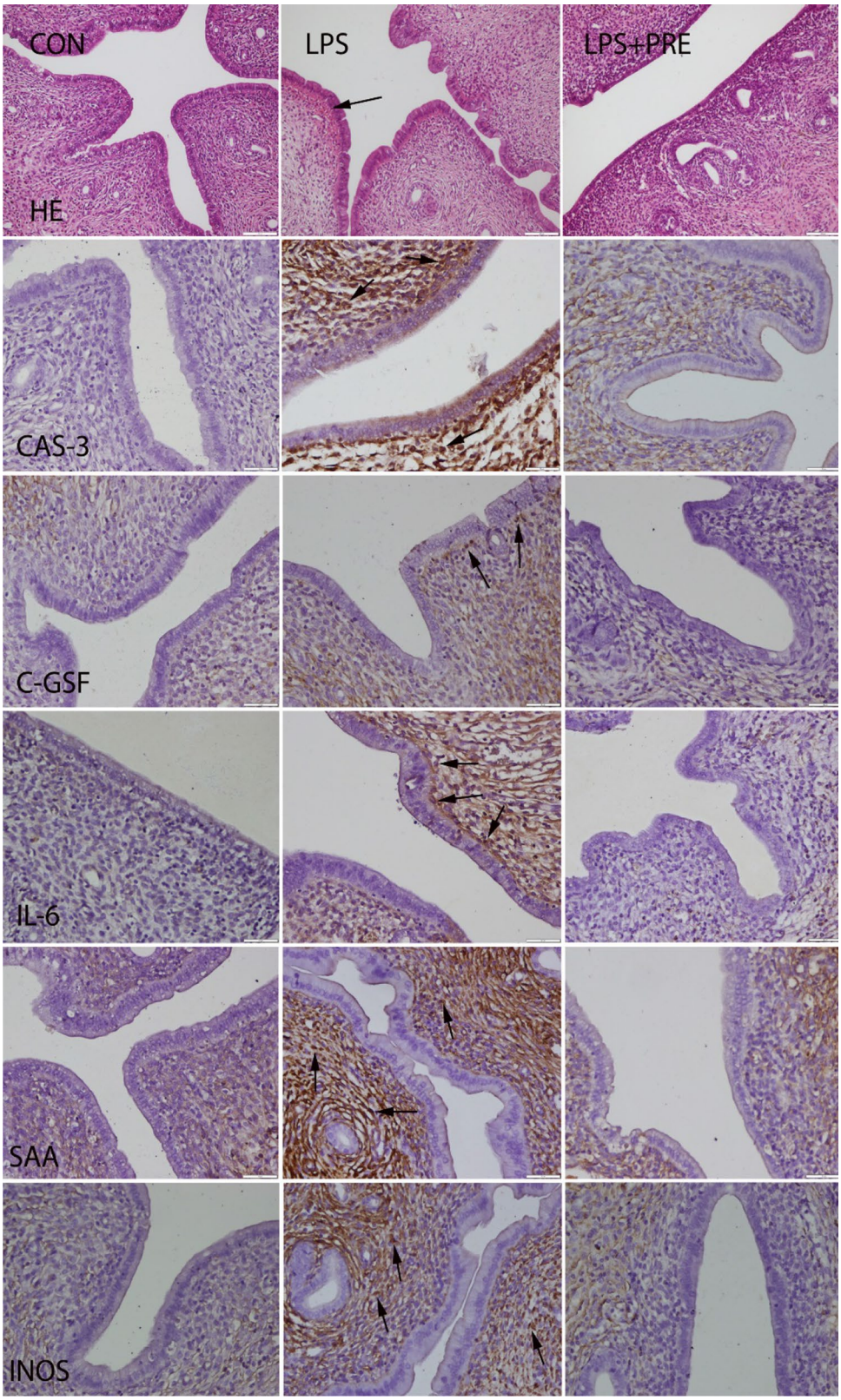

Fig. 3 Endometrial histopathology: normal endometrial architecture in C group; hemorrhage (arrow) in submucosa of the endometrium (C). No pathological findings in SP group, H&E. Endometrial immunohistochemistry: second row; Cas-3, third row: G-CSF, fourth row: IL-6, fifth row: SAA and sixth row: iNOS immunoreaction of the endometrium: no expression in C group, numerous immunopositive reaction in stromal cells (arrows) in S group, decreased immunoreaction in SP group, streptavidin biotin peroxidase method, bars = 50 μ

of iNOS in LPS-induced sepsis in all tissues except for urinary bladder. Cervical iNOS inhibition with PGB was better than in C group. Similarly, ameliorative effects of PGB on tuba uterina, uterus and ovarian tissues in terms of iNOS immunochemical staining showed as good results as in the control group.

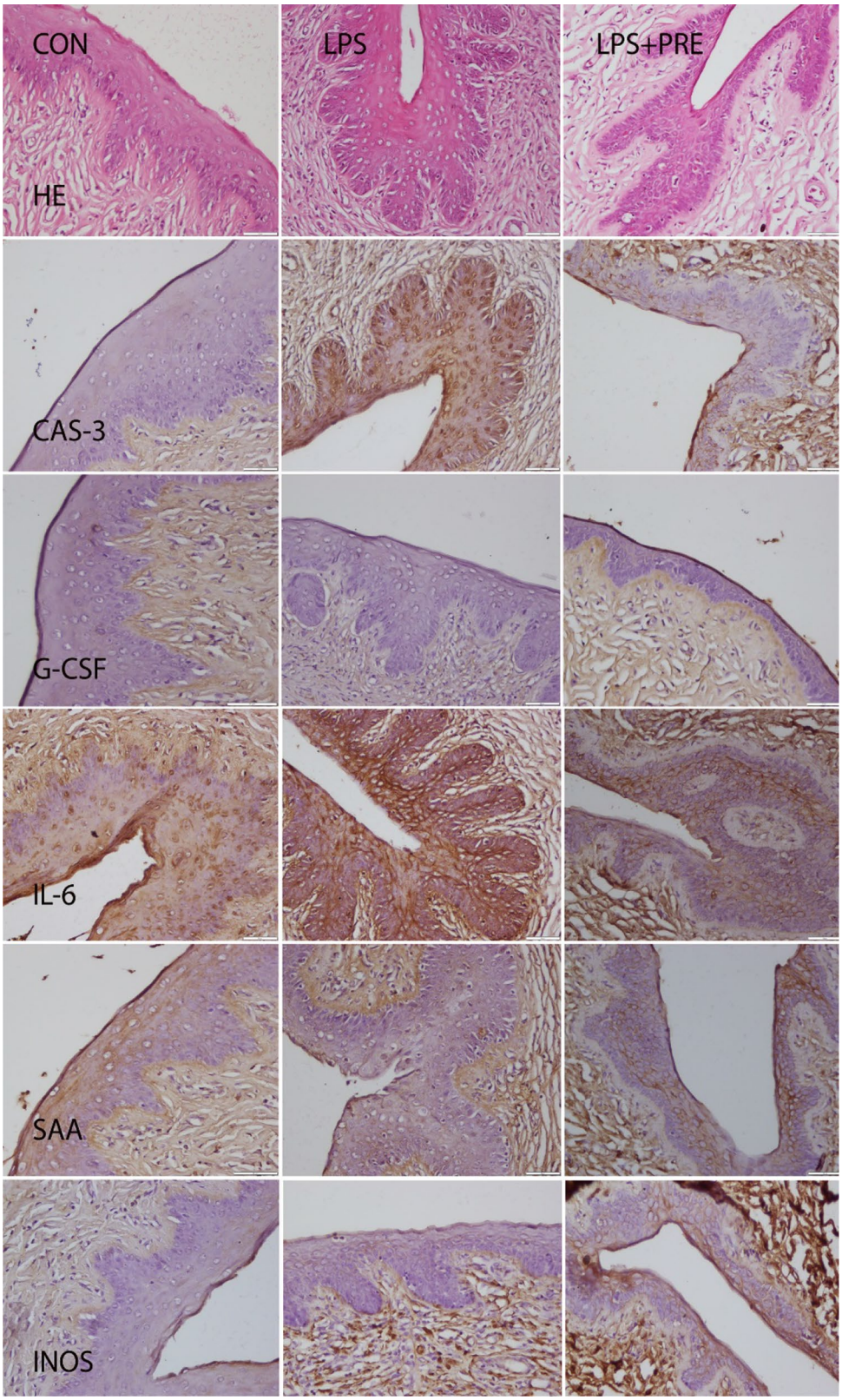

Fig. 4 Uterine cervical histopathology: normal cervical mucosa in C, S and SP group, H&E. Cervical immunohistochemistry: second row; Cas-3, third row: G-CSF, fourth row: IL-6, fifth row: SAA and sixth row: iNOS immunoreaction of the cervix: negative to slight expression in C group; increased reaction in S group, decreased immunoreaction in SP group, streptavidin biotin peroxidase method, bars = 50 µ

Kumar et al. found that neutrophils iNOS expression, NO content, plasma nitrite and cytokines played an important role in the assessment of the severity of sepsis and organ toxicity [10]. iNOS represents oxidative effects. Takatani et al. demonstrated that iNOS during the late phase of sepsis was associated with hypothermia and immune cell

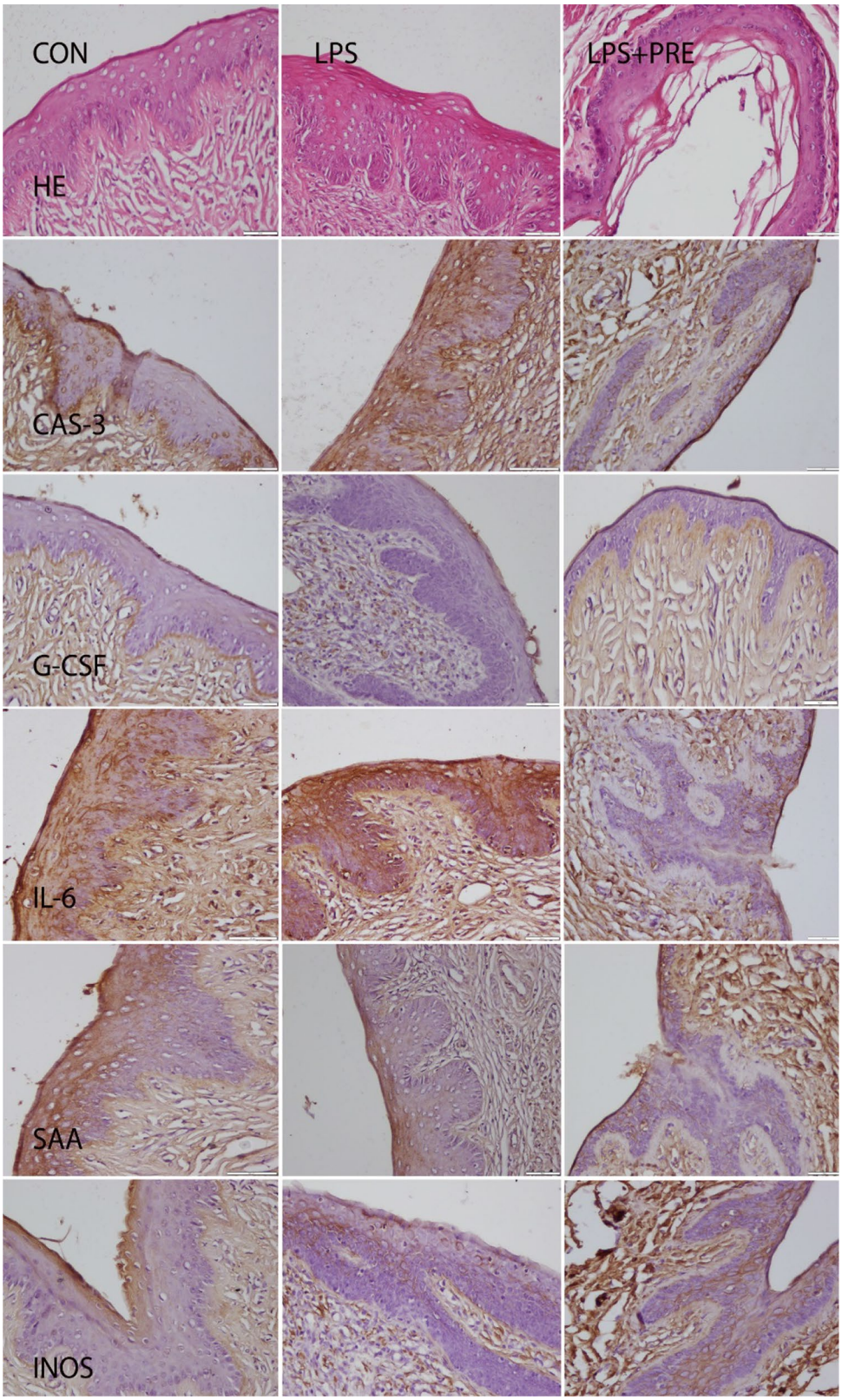

Fig. 5 Vaginal histopathology: normal vaginal mucosa in control, S and SP group, H&E. Vaginal immunohistochemistry: second row; Cas-3, third row: G-CSF, fourth row: IL-6, fifth row: SAA and sixth row: iNOS immunoreaction of the vagina: very slight expression in C group; increased reaction in S group, decreased immunoreaction in SP group, streptavidin biotin peroxidase method, bars = 50 μ

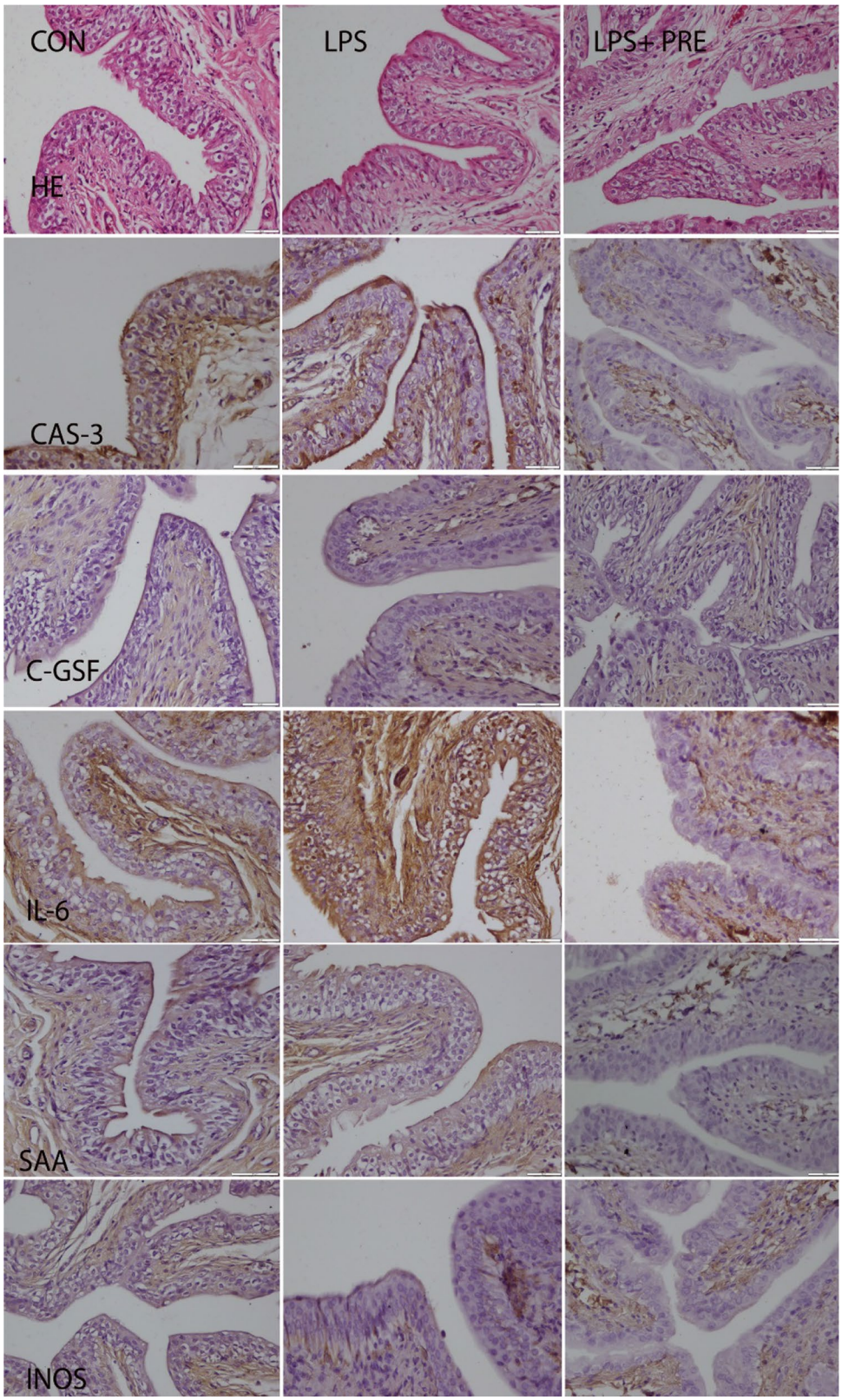

Fig. 6 Urinary bladder histopathology: normal urinary bladder histology in C, S and SP group, H&E. Bladder immunohistochemistry: second row; Cas-3, third row: G-CSF, fourth row: IL-6, fifth row: SAA and sixth row: iNOS immunoreaction of the urinary bladder: Similar immune expression in all markers except IL-6. Streptavidin biotin peroxidase method, bars = 50 μ

migration, and iNOS inhibition could provide a therapeutic benefit [36]. According to these results, we should emphasize that PGB has the protective effects on uterine, tubal, ovarian, vaginal and cervical tissues (providing better results than in C group) in sepsis survivors.

Table 4 Statistical analysis of immunohistochemically positive cell numbers

Markers	Organs	Control (C)	Sepsis (S)	Sepsis + Pregabalin (SP)	*p* value in among groups
Caspase-3	Ovarium	1.25 ± 0.36	19.25 ± 0.72	7.50 ± 0.56	C-S < 0.001 C-SP < 0.001 S-SP < 0.001
	Tuba U	1.87 ± 0.51	8.12 ± 0.39	4.12 ± 0.51	C-S < 0.001 C-SP < 0.05 S-SP < 0.001
	Uterus	2.00 ± 0.56	20.00 ± 1.10	7.62 ± 0.46	C-S < 0.001 C-SP < 0.001 S-SP < 0.001
	Cervix	1.00 ± 0.37	13.12 ± 1.44	6.75 ± 0.77	C-S < 0.001 C-SP < 0.01 S-SP < 0.001
	Vagina	1.25 ± 0.36	6.62 ± 0.37	1.37 ± 0.46	C-S < 0.001 C-SP-NS S-SP < 0.001
	Urinary bladder	8.12 ± 0.39	9.00 ± 0.70	8.50 ± 0.53	C-S- NS C-SP-NS S-SP-NS
G-CSF	Ovarium	0.25 ± 0.16	10.00 ± 0.59	0.62 ± 0.26	C-S < 0.001 C-SP-NS S-SP < 0.001
	Tuba U	0.00 ± 0.00	3.87 ± 0.54	0.37 ± 0.18	C-S < 0.001 C-SP-NS S-SP < 0.001
	Uterus	4.25 ± 0.52	19.50 ± 1.40	7.75 ± 0.59	C-S < 0.001 C-SP < 0.05 S-SP < 0.001
	Cervix	0.25 ± 0.16	2.62 ± 0.49	1.12 ± 0.12	C-S < 0.001 C-SP-NS S-SP < 0.05
	Vagina	0.50 ± 0.26	9.37 ± 0.49	1.37 ± 0.46	C-S < 0.001 C-SP-NS S-SP < 0.001
	Urinary bladder	3.37 ± 0.32	4.00 ± 0.53	3.75 ± 0.45	C-S-NS C-SP-NS S-SP-NS
IL-6	Ovarium	0.25 ± 0.16	10.75 ± 0.59	4.75 ± 0.36	C-S < 0.001 C-SP < 0.001 S-SP < 0.001
	Tuba U	0.12 ± 0.12	8.00 ± 0.42	0.87 ± 0.29	C-S < 0.001 C-SP-NS S-SP < 0.001
	Uterus	0.37 ± 0.26	11.62 ± 0.65	1.62 ± 0.32	C-S < 0.001 C-SP-NS S-SP < 0.001
	Cervix	3.87 ± 0.71	10.37 ± 1.40	2.37 ± 0.46	C-S < 0.001 C-SP-NS S-SP < 0.001
	Vagina	0.25 ± 0.16	9.12 ± 0.63	1.12 ± 0.29	C-S < 0.001 C-SP-NS S-SP < 0.001
	Urinary bladder	8.50 ± 1.08	13.87 ± 1.25	7.50 ± 0.75	C-S < 0.05 C-SP-NS S-SP < 0.001

Table 4 (continued)

Markers	Organs	Control (C)	Sepsis (S)	Sepsis + Pregabalin (SP)	*p* value in among groups
SAA	Ovarium	0.25 ± 0.16	15.62 ± 1.22	3.50 ± 0.37	C-S < 0.001 C-SP < 0.05 S-SP < 0.001
	Tuba U	0.00 ± 0.00	3.62 ± 0.32	0.75 ± 0.25	C-S < 0.001 C-SP-NS S-SP < 0.001
	Uterus	0.75 ± 0.31	19.25 ± 0.61	2.12 ± 0.47	C-S < 0.001 C-SP-NS S-SP < 0.001
	Cervix	2.12 ± 0.39	11.25 ± 1.53	6.50 ± 0.68	C-S < 0.001 C-SP < 0.05 S-SP < 0.01
	Vagina	0.25 ± 0.16	6.50 ± 0.42	0.75 ± 0.31	C-S < 0.001 C-SP-NS S-SP < 0.001
	Urinary bladder	1.62 ± 0.32	2.00 ± 0.37	2.00 ± 0.50	C-S-NS C-SP-NS S-SP-NS
iNOS	Ovarium	0.00 ± 0.00	11.25 ± 1.35	0.62 ± 0.26	C-S < 0.001 C-SP-NS S-SP < 0.001
	Tuba U	1.00 ± 0.26	9.87 ± 0.83	0.87 ± 0.35	C-S < 0.001 C-SP-NS S-SP < 0.001
	Uterus	2.25 ± 0.64	16.62 ± 2.18	6.00 ± 0.59	C-S < 0.001 C-SP-NS S-SP < 0.001
	Cervix	2.37 ± 0.26	4.37 ± 0.56	1.25 ± 0.36	C-S < 0.01 C-SP-NS S-SP < 0.001
	Vagina	1.37 ± 0.46	10.37 ± 0.32	3.62 ± 0.41	C-S < 0.001 C-SP < 0.01 S-SP < 0.001
	Urinary bladder	1.50 ± 0.18	1.75 ± 0.36	1.87 ± 0.17	C-S-NS C-SP-NS S-SP-NS

NS non-significant, values presented as mean ± SE; one-way ANOVA Bonferroni test, *GCSF* granulocyte colony-stimulating factor, *IL-6* interleukin-6, *iNOS* inducible nitric oxide synthase, *SAA* Serum Amyloid A

The doses of PGB used in the medical literature generally ranged from 50 to 750 mg/day [27]. Asgari et al. demonstrated that oral administration of 150 mg PGB was an effective and safe method (better pain relief and lower side effects) for postoperative pain management after laparoscopic hysterectomy [25].

Such dosages of PGB are prevalent in the medical literature, but our study differs from all of them, we wanted to attract attention to the use of PGB as a protective agent for female patients with sepsis. We used the dose of 30 mg/kg (p.o.) of PGB as a protective agent in the current study.

PGB has other advantages such as no interaction with many drugs (including hypoglycemic agents, diuretics, insulin). For this reason, we preferred it as a protective agent in this study. This feature may be very important due to the different phases of sepsis and the necessity to use multiple drugs. In addition, adding PGB to the routine sepsis treatment may be useful due to its protective effects.

In our study, we concluded that in SP group, all the markers representing oxidative stress (TOS, OSI, Caspase-3, G-CSF, IL-6, SAA and iNOS), inflammatory response and apoptosis were improved with the similar result as in C group (maximal effect was seen in tuba uterina). These data can predict that tubal protection with PGB can protect from the future possible ovarian cancers in sepsis survivors because presently it is known that the majority of high-grade serous ovarian cancers evolve from the fallopian tube epithelium [37]. Hence, it is reasonable

to assume that the use of PGB medication in addition to standard sepsis therapy will allow to protect from several urogenital malignancies such as epithelial ovarian cancer (EOC) mentioned above.

Sepsis is a serious cause of mortality and a life-threatening disorder not only for adults but also for newborns and infants [2]. In this context, we need to emphasize that the protection from the damage of sepsis is important especially for this age group for the purpose of maintaining a healthy life in the future.

Conclusion

Based on the results of this study, we think that PGB administration in sepsis is safe for patients and its use is convenient in clinical trials and treatment of certain detrimental diseases. Supplementation of some protective agents such as PGB to standard sepsis treatment may be helpful for the health of the female reproductive system, prevention of genital cancers and infertility.

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Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest with respect to the authorship and publication of this article.

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