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GENERAL GYNECOLOGY

Overall systematic approach to sepsis damages on urogenital tissues: protective power of lacosamide

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Abstract

Purpose The aim of the study was to evaluate the harmful effects of sepsis on the urogynecological tissues and the ability of Lacosamide (LCM) on Lipopolysaccharide (LPS)-induced cytokine production, oxidative stress and apoptotic pathways, in the experimental rat sepsis model.

Methods Twenty-four female Wistar albino rats (12 months old) were divided into 3 groups as follows: control group (Group I) (0.1 ml/oral and i.p. saline, single dose), sepsis group (Group II) (5 mg/kg LPS, i.p. single dose) and sepsis + LCM group (Group III) (5 mg/kg LPS, i.p. single dose and 40 mg/kg LCM). Six hours after the last LPS administration, the animals were sacrificed. Subsequently, the analyses of urogenital tissues total oxidant/antioxidant status, histopathological and immunohistochemical analyses were performed.

Results Total oxidant capacity (TOC) and oxidative stress index (OSI) values in the urogenital tissues were increased in the urogenital tissues in Group II [Total antioxidant capacity (TAC) was decreased] compared to group I ($p < 0.05$). LCM improved these values ($p < 0.05$). The immunohistochemical markers (Tumor Necrosis Factor-alpha (TNF-α), interleukin-1 beta (IL-1β), heat shock protein 70 (HSP-70), C-reactive protein (CRP), Malondialdehyde (MDA) were significantly increased in Group II ($p < 0.001$). With the administration of LCM (Group III), the expressions of above-mentioned markers were markedly decreased ($p < 0.001$). Marked hyperemia and slight hemorrhages with neutrophil leukocyte infiltrations were seen histopathologically in Group II. LCM treatment ameliorated the pathological findings.

Conclusion These findings demonstrated that sepsis caused oxidative stress, apoptosis and inflammation in the urogenital tissues. We revealed that LCM ameliorated the damage caused by sepsis in urogenital tissue.

Keywords Urogenital system · Lipopolysaccharide · Sepsis · Lacosamide · Immunohistochemistry

Introduction

Today, sepsis is still a severe and deadly public health issue that includes physiological, pathological, and biochemical abnormalities following the infection. According to a recent universally accepted term "pathobiology", sepsis affects several systems and disciplines such as life-threatening multiple organ dysfunction, cell biology, biochemistry, and immunology. Although due to the difficulties of data collection, there are no clear data, lastly, Sakr et al. revealed that more than 10,000 patients from 730 ICUs (intensive care units), indicate that approximately 30% of all ICU patients have sepsis [1808 patients (18.0%) already sepsis at ICU admission], as defined by the presence of infection and organ dysfunction. They also revealed that occurrence rates of sepsis varied from 13.6 to 39.3% in the different regions. Overall ICU and hospital mortality rates were 25.8% and 35.3%, respectively, in patients with sepsis, but it varied from 11.9% and 19.3% (Oceania) to 39.5% and 47.2% (Africa), respectively [1]. Nevertheless, the global mortality rate related to sepsis is even higher than the combination of breast cancer, prostate cancer and HIV-AIDS rates [2].

Therefore, despite all the intensive treatment methods and preventive efforts, it is still a serious illness which is a leading

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cause of mortality that causes cost-intensive care [3]. In case of a minimal doubt occurring in a patient about the infection, at the time of diagnosis, a moderate organ dysfunction and more than 10% of hospital mortality may already exist [4].

Lipopolysaccharide (LPS) (also known as endotoxin) is a fundamental structural component of the outer membrane of the cell wall of Gram-negative bacteria, and all pathophysiological processes are initiated via macrophage activation which is triggered by LPS. The other innate immune cells are B lymphocytes, T lymphocytes, and mast cells, apart from activated macrophages. In early stages of sepsis (especially in the first 4 h), a massive and uncontrolled released of cytokines into the bloodstream occurs consequent to infection such as Tumor necrosis factor-alpha (TNF-α), IL-1-α, IL-1β, IL-6, IL-10, Granulocyte-Colony stimulating factor (G-CSF), Serum Amyloid A (SAA), Heat shock protein 70 (HSP-70), C-reactive protein (CRP), inducible nitric oxide synthase (iNOS), Malonaldehyde (MDA), Nitric oxide (NO), Caspase-3, and free oxygen radicals [5–9]. As a result, overall cytokine response causes vascular damage, intravascular coagulation, systemic vasodilation, vasoplegia, hyporesponsiveness to vasoconstrictors, hypoperfusion, multiple organ dysfunction syndrome (MODS), septic shock and finally, serious morbidity and death [7].

Lacosamide (LCM, i.e., R-2-acetamido-N-benzyl-3 methoxypropionamide) is a new synthesized amino acid which is used as an antiepileptic agent. It was approved in 2008 for the treatment of several neurological diseases which initially included partial-onset seizures, and then, temporal lobe epilepsy, focal impaired awareness seizures, complex partial seizures, secondarily generalized seizures, and bilateral tonic–clonic seizures [10, 11].

LCM is different from traditional sodium channel blockers because it acts mainly on the slow activation state in sodium channels [12]. The use of LCM in different indications due to its relatively wide therapeutic window and minimal drug–drug interactions has become more extensive in recent years [13].

In the current study, we investigated whether LCM with its proven inflammatory and antioxidant features has a protective or therapeutic effect on experimental LPS-induced female rat sepsis model. For this purpose, we thoroughly studied sepsis effects on urogenital area with the help of biochemical assays of oxidative stress (TAC, TOC, OSI), apoptosis assays, histopathological characteristics as well as our preferred immunohistochemical staining (TNF-α, HSP-70, CRP, IL-1β, MDA).

Materials and methods

Experimental design

Twenty-four female Wistar Albino rats (12 months old) were obtained from the Experimental Animal Production and the Experimental Research Laboratory of Burdur Mehmet Akif Ersoy University (Burdur, Turkey). The procedures performed on rats were reviewed and approved by the Animal Experiments Local Ethics Committee of Burdur Mehmet Akif Ersoy University (Ethic No: 308, August 2017/02).

The animals were kept under standard laboratory conditions (temperature, 22–24 °C, humidity, 55–60% and 12-h light/dark cycles). All groups received ad libitum feeding of standard commercial chow diet (Korkuteli yem) and tap water. Other environmental factors were kept the same in all groups. Before the experiment began, the animals were monitored for 7 days and adjusted to their orientation. One Eurostandard type IV cage was used for every eight rats.

The rats were randomly divided into three groups of eight rats each as follows: Group I (control), Group II (exposure to LPS) and Group III (LCM treatment group). Group I was given standard commercial chow and tap water, saline was administered orally in equivalent volume to LCM and intraperitoneally in equivalent volume to LPS. Rats in group II were given as 5 mg/kg LPS (i.p. lipopolysaccharide, 500 mg flk, 048K4126, Sigma-Aldrich, USA) in a single dose together with LCM equivalent volume of saline. Group III received LPS [5 mg/kg, i.p. (into the right groin region), single dose] and LCM [40 mg/kg, i.p. (into the left groin region) (VIMPAT® Lacosamide film tablet)] daily for 3 days. Last LCM dose was administered 30 min before LPS. LCM was dissolved in normal saline. Animals were sacrificed by Ketamine (80–100 mg/kg, 0.2–0.5 ml, i.p. single dose) and Xylazin (6–8 mg/kg, 0.1–0.3 ml, i.p. single dose) 6 h after the last LPS administration, following the standardized ethical procedures. Blood samples were collected from each animal for biochemical analyses. Urogenital tissues were rapidly dissected. Ovary, fallopian tube, uterus, cervix, vagina, urinary bladder and urethral tissues were kept in 10% formalin solution for histological and immunohistochemical examinations. The tissues were homogenized and stored at − 80 °C until analyzed for oxidant/antioxidant (TAC-TOC-OSI) status.

Biochemical analyses

The urogenital tissues extracted from the sacrificed 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). Subsequently, the tissue samples were centrifuged at 10,000*g* for 10 min at + 4 °C. The automated colorimetric method developed by Erel was used for measuring TAC and TOC levels in tissue samples [14]. The absorbance change was measured at 660 nm using a spectrophotometer (Shimadzu UV1601 spectrophotometer, Japan) and the results were expressed as mmol Trolox Eq/mg protein. OSI was defined by the formula

OSI (arbitrary unit) = [(TOC, mmol/L)/(TAS, mmol Trolox equivalent/L)/100].

Histopathological examinations

Genito-urinary system organs (ovary, fallopian tube, uterus, cervix, vagina, urinary bladder and urethra) were grossly examined, the tissue samples were collected and fixed in 10% neutral formalin during the necropsy. Tissue samples were routinely processed by an automatic tissue processor equipment (Leica® ASP300S, Wetzlar, Germany), then embedded in paraffin and 5-µm sections were taken by a Leica RM2155 rotary microtome (Leica Microsystems®, Wetzlar, Germany). Tissue sections were stained with hematoxylin–eosin (H&E) and examined under the light microscope. Histopathological changes were graded in a blinded manner.

Immunohistochemical examinations

After histopathological examination serial sections were immunostained with MDA [Anti-Malondialdehyde antibody (ab6463)], C-reactive protein [Anti-C-Reactive Protein antibody − Aminoterminal end (ab65842)], heat shock protein 70 [Anti-Hsp70 antibody [5A5] (ab2787)], IL-1β [Anti-IL1 beta antibody (ab2105)] and TNF-α [Anti-TNF alpha antibody (ab6671)] by streptavidin biotin technique. All primary and secondary antibodies were purchased from Abcam (Cambridge, UK).

The sections were incubated with 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 secondary antibody. The antigens were demonstrated using diaminobenzidine (DAB) as the chromogen. Positive controls were used for each marker. For negative controls 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 in every section at X40 magnification in all groups. The 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, ANOVA and post hoc Bonferroni and 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

Biochemical analyses

Ovarian tissue

There were significant changes in TAC, TOC, and OSI levels ($p < 0.05$) in ovarian tissues among the groups. A statistically significant increase in TOC levels ($p = 0.036$) and OSI index ($p = 0.028$) was observed in group II compared to group I. TAC levels were significantly increased in group III compared to group II ($p = 0.004$). However, a significant decrease was observed in group III compared to group II in terms of TOC levels ($p = 0.003$) and OSI ($p = 0.001$) (Table 1).

Fallopian tubes

There were significant changes in TOC levels ($p < 0.05$), but not in TAC and OSI levels ($p > 0.05$) in fallopian tube tissues was observed among the groups. A significant increase in TOC levels in fallopian tube tissues ($p = 0.012$) was observed in group II compared to group I. A significant decrease in TOC levels ($p = 0.031$) was observed in group III compared to group II (Table 2).

Endometrial tissue

There were significant changes in TAC, TOC, and OSI levels ($p < 0.05$) in the endometrial tissue among the groups. A statistically significant increase in TOC levels ($p = 0.002$) and OSI index ($p = 0.002$) was observed in group II compared to group I. A statistically significant decrease in TOC ($p = 0.043$) and OSI ($p = 0.009$) levels was observed in group III compared to group II. However, a significant decrease in TAC levels ($p = 0.037$) was observed in group II compared to group I, and a significant increase in TAC levels ($p = 0.0003$) was observed in group III compared to group II (Table 3).

Histopathological and immunohistochemical analyses

Gross examination of genitourinary system revealed that normal appearance in control and LCM-treated group while slight to moderate hyperemia was observed in LPS group. The histopathological examination revealed no pathological findings in the examined genitourinary system organs in the control group. Marked hyperemia and sometimes slight hemorrhages with neutrophil leukocyte infiltrations were seen in organs in LPS group. LCM treatment ameliorated the pathological findings. The immunohistochemical examination showed that LPS caused an increase in TNF-α, HSP-70, CRP, IL-1β, and MDA expressions in all examined organs. LCM caused marked amelioration both histopathological and immunohistochemical findings (Figs. 1, 2, 3, 4, 5, 6, 7). The results of the statistical analysis immunohistochemically positive cell numbers are shown in Table 4.

Table 1 Oxidative stress markers of Ovarian tissues

Groups	TAS (mmol Trolox equiv./L)		TOS (μmol H_2O_2 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 ± 0.15[ab]	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

Table 2 Oxidative stress markers of fallopian tubes

Groups	TAS (mmol Trolox equiv./L)		TOS (μmol H_2O_2 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

Table 3 Oxidative stress markers of Endometrium

Groups	TAS (mmol Trolox equiv./L)		TOS (μmol H_2O_2 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[ab]	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

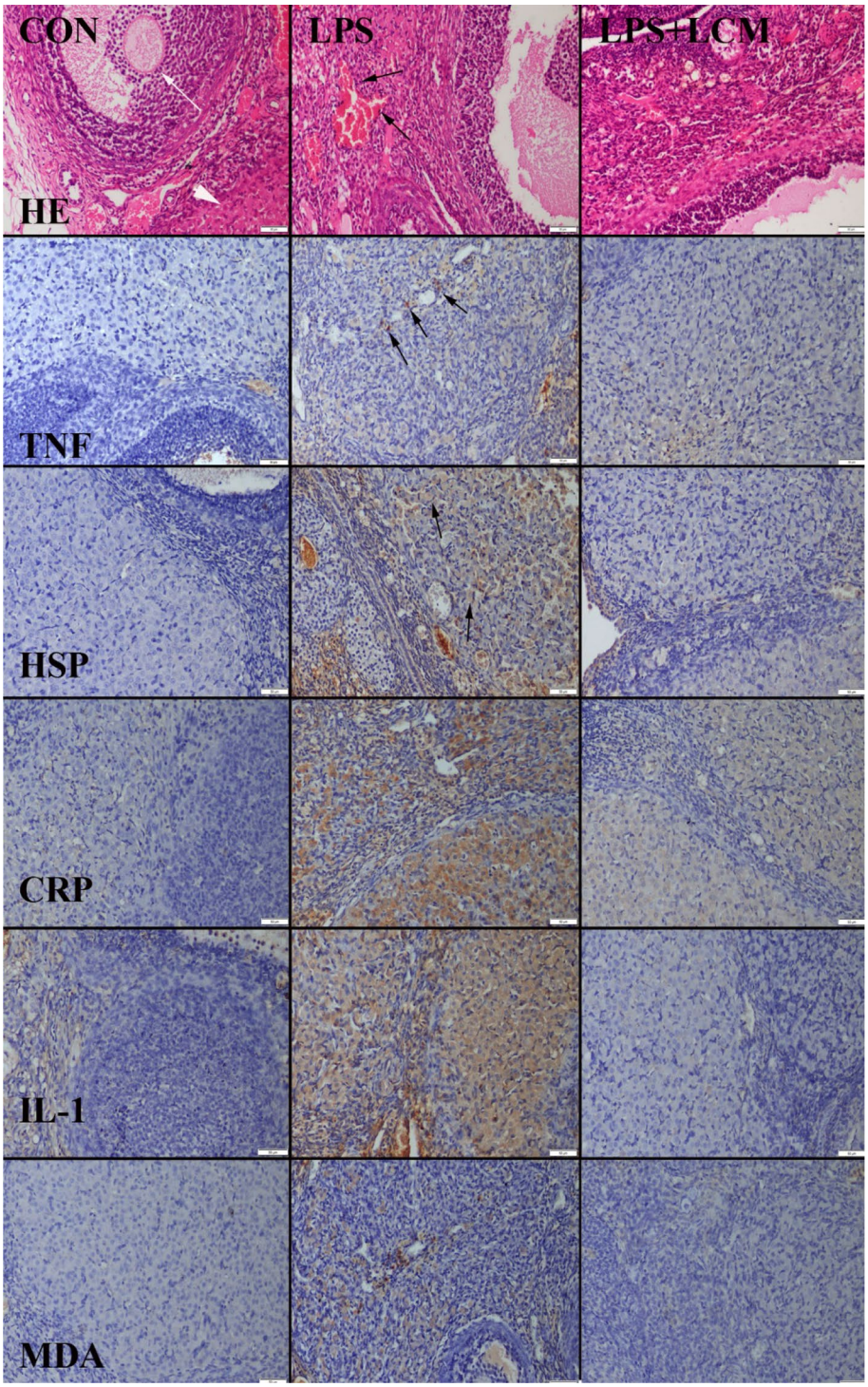

Fig. 1 Ovarial histopathology: first row: normal ovarial histology, oocyst (white arrow) and corpus luteum (white arrow head) in control group; severe hyperemia and neutrophil chemotaxis (arrows) to the ovary in LPS group, almost normal histology in LCM-treated group, hematoxylin and eosin, bars = 100 µ. Second row; TNF-α, third row: HSP-70, fourth row: CRP, fifth row: IL-1 and sixth row: MDA immunoreaction. No expression in control group; numerous immunopositive cells (arrows) in corpus luteum in LPS group, slight or negative reaction in LCM-treated group, streptavidin–biotin peroxidase method, bars = 50 µ

Discussion

To our knowledge, our study is the first and unique original research investigating the efficacy of LCM on septic urogenital tissues in LPS-induced female rat sepsis model. Nevertheless, although many different agents and tissues were studied pertinent to sepsis in the literature, our results are similar to those studies in terms of inflammatory markers and ameliorative effects of the variety of agents on sepsis. In addition, in the literature, a lot of markers, including those used in our study, were examined mostly in serum [8, 15–18]. But, differently, in our study all the tissue markers were predominantly examined immunohistochemically to evaluate the effects of sepsis on urogenital tissues.

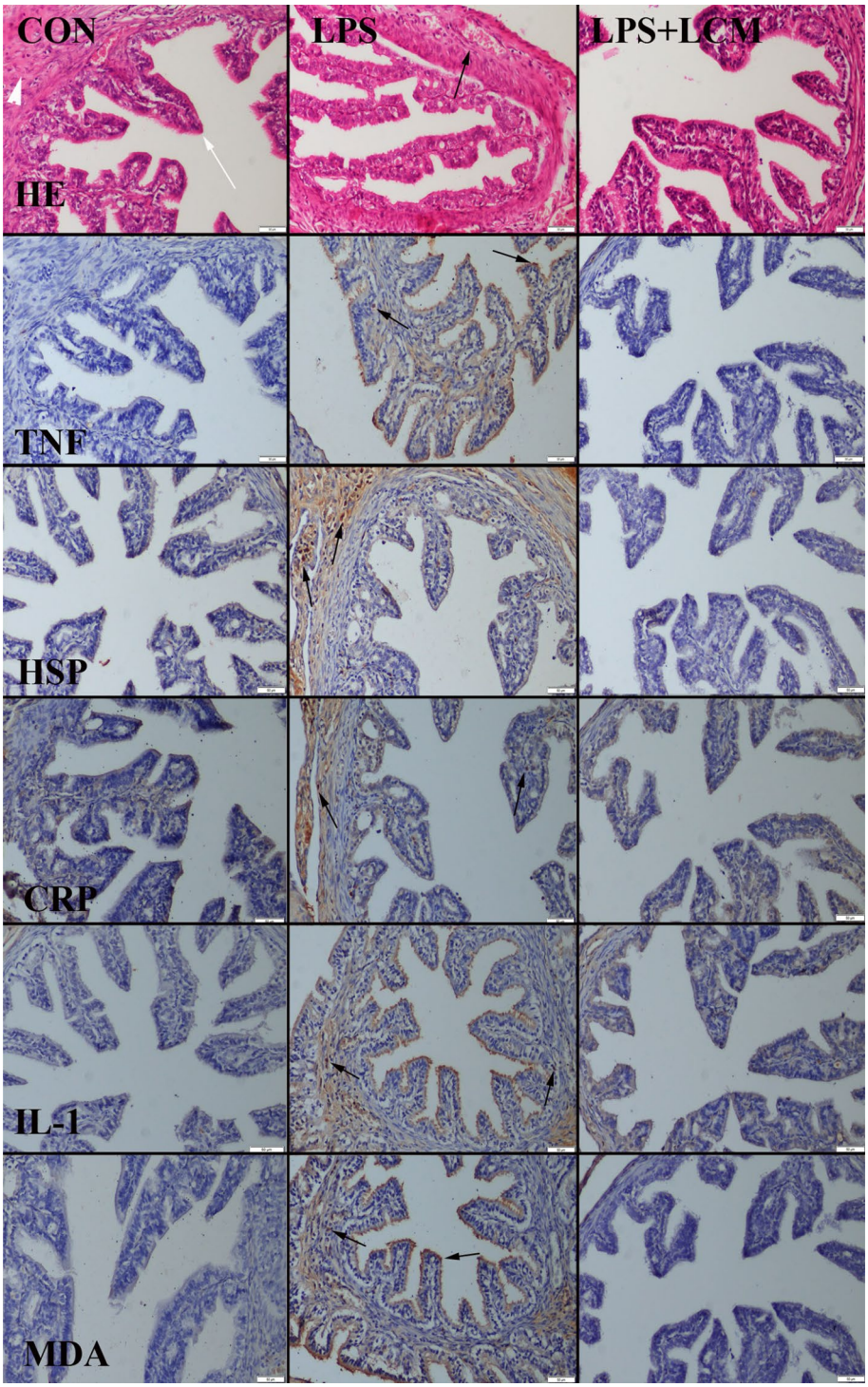

Fig. 2 Fallopian tube histopathology: normal fallopian tube architecture, epithelial layer (white arrow) and muscularis mucosae (white arrow head) in control group; severe hyperemia in submucosa (arrow), no pathological findings in LPS-treated group, hematoxylin and eosin. Tubal immunohistochemistry: second row; TNF-α, third row: HSP-70, fourth row: CRP, fifth row: IL-1 and sixth row: MDA immunoreaction of the fallopian tube. Negative expression in control group; increased immunopositive reaction in the fallopian tube cells (arrows) in LPS group, no or very slight reaction in LPS-treated group, streptavidin–biotin peroxidase method, bars = 50 µ

In one multicenter human study evaluating oxidative stress, Lorente et al. revealed that elevated MDA serum levels represent an unbalanced oxidant state and are related with poor prognosis in patients with severe sepsis [15]. In another study, El-Awady et al. demonstrated that Agmatine (AGM), the endogenous metabolite of L-arginine, significantly reduced oxidative stress, CRP, MDA (aortic), iNOS and nitric oxide levels [8]. In our research, we studied other markers namely TAC, TOC, and OSI levels [14] in ovarian, fallopian tube and endometrial tissues for evaluating the oxidative stress. In this context, we revealed that TOC and OSI levels were markedly decreased (together with concurrent TAC increase) with the administration of LCM in ovarian and endometrial tissues. In fallopian tube, only TOC levels

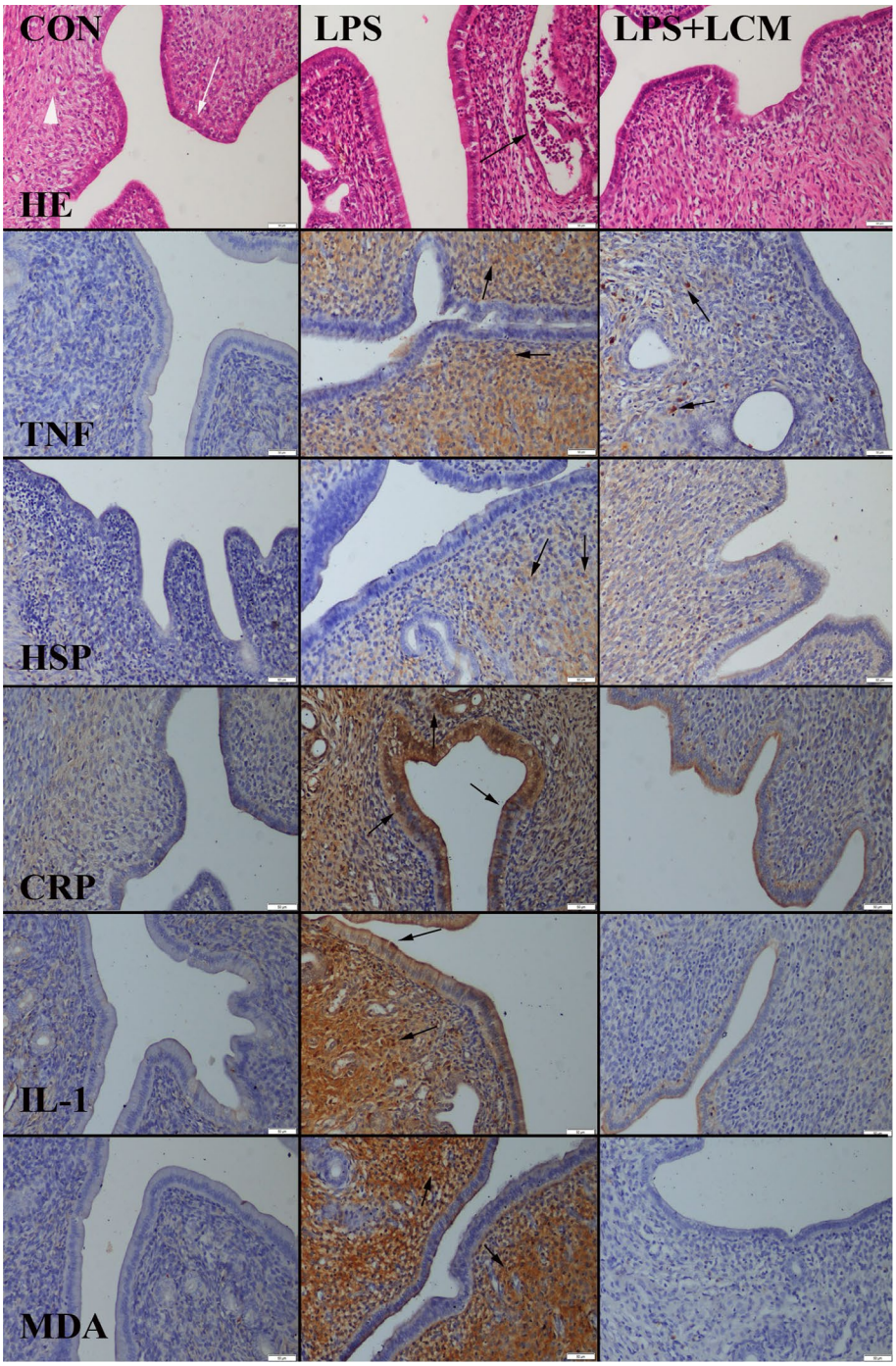

Fig. 3 Endometrial histopathology: normal endometrial architecture, epithelial layer (white arrow) and propria mucosae (white arrow head) in control group; neutrophil infiltrations at the endometrium (arrow), no pathological findings in LPS-treated group, hematoxylin and eosin. Endometrial immunohistochemistry: second row; TNF-α, third row: HSP-70, fourth row: CRP, fifth row: IL-1 and sixth row: MDA immunoreaction of the uterus: no expression in control group, numerous immunopositive reaction in stromal cells (arrows) in LPS group, marked decreased immunoreaction in LPS-treated group, streptavidin–biotin peroxidase method, bars = 50 µ

were significantly decreased by LCM, but there was no significance in the values of TAC and OSI. In another human research, Annagür et al. demonstrated that TAC levels in the group receiving conventional sepsis treatment were significantly higher than in the group receiving no treatment. Whereas, post-treatment levels of TOS, OSI and Paraoxonase-1 (PON-1) in the sepsis group were not significantly different compared to the control group. According to these results, neither TOC nor OSI levels were ameliorated with the standard neonatal sepsis therapy [19]. Unlike this study, LCM ameliorated TAC, TOC and OSI levels in our research. These data demonstrate the superior potential effects of LCM on urogenital tissues affected by sepsis compared to the standard sepsis treatment. Due to no information in the literature evaluating the urogenital tissues in terms of oxidative stress, our results are extremely important considering the hope to elucidate this subject.

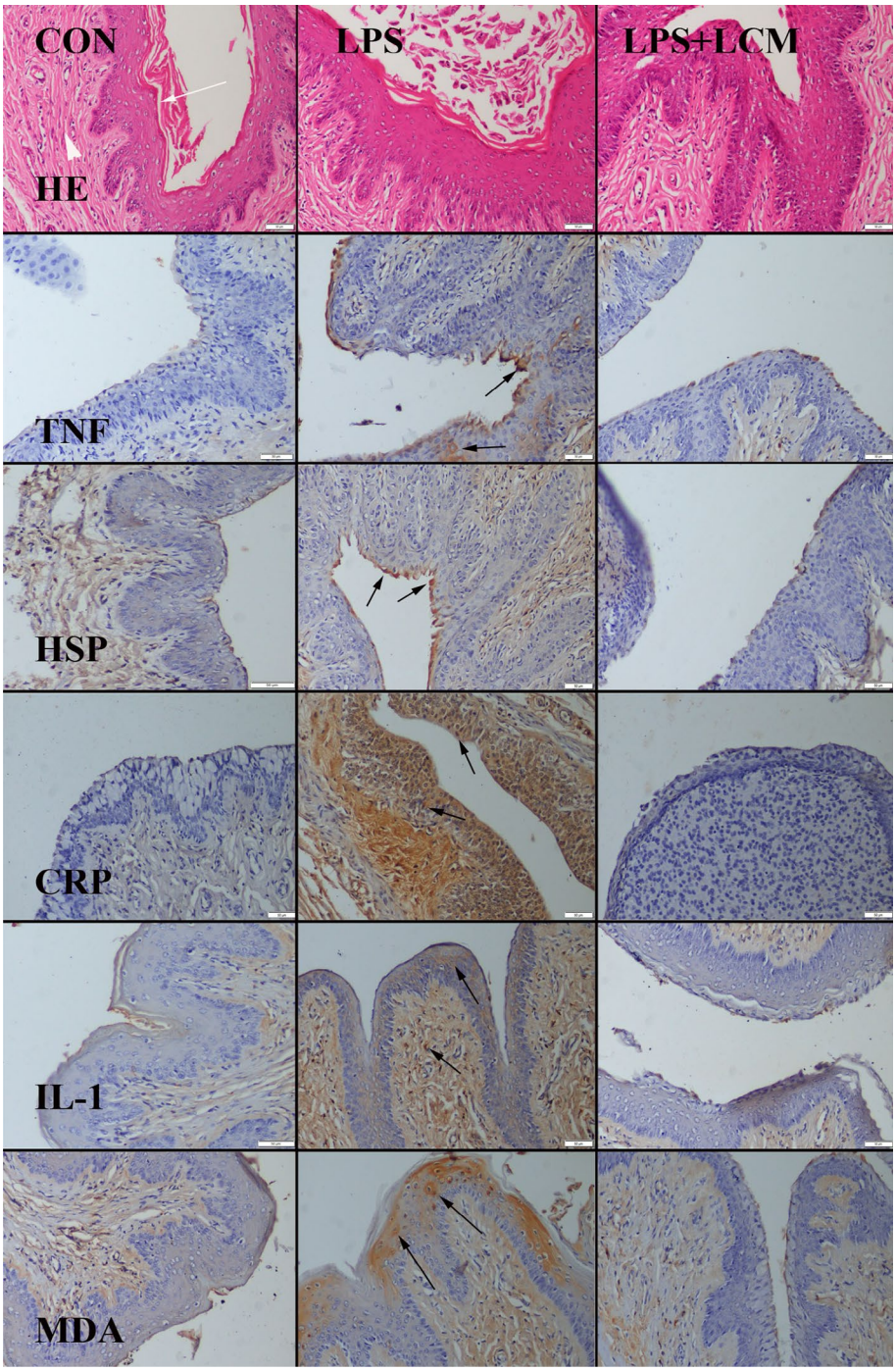

Fig. 4 Cervical histopathology: normal cervical mucosa, epithelium (white arrow) and propria mucosae (white arrow head) in control and LCM-treated groups, epithelial desquamation in LPS group, hematoxylin and eosin. Cervical immunohistochemistry: second row; TNF-α, third row: HSP-70, fourth row: CPR, fifth row: IL-1 and sixth row: MDA immunoreaction of the cervix: negative to slight expression in control group; increased reaction in LPS group, decreased immunoreaction in LCM-treated group, streptavidin–biotin peroxidase method, bars = 50 μ

On the other hand, in a unique study which explored the sepsis effects on uterus, Aksoy et al. showed that significantly increased serum IL-6 and TNF-α levels (pg/ml) and elevated MDA concentrations representing oxidative stress, in uterine, liver and kidney tissues were found in the ovariectomized rat sepsis group, and all these results were ameliorated with progesterone administration [16]. Similarly, we also revealed that IL-1β, TNF-α, and MDA immunochemical positiveness were high in all septic urogenital tissues including uterus. All the damage induced by sepsis was prevented by LCM in our study. In conclusion, though the agents used in two studies were different, but, generally, our results are similar to studies showing and proving beneficial effects by any drug or substance on any tissue exposed to sepsis. In this context, our results were consistent with Aksoy's study, in terms of tissue protective effect and physiopathological mechanism.

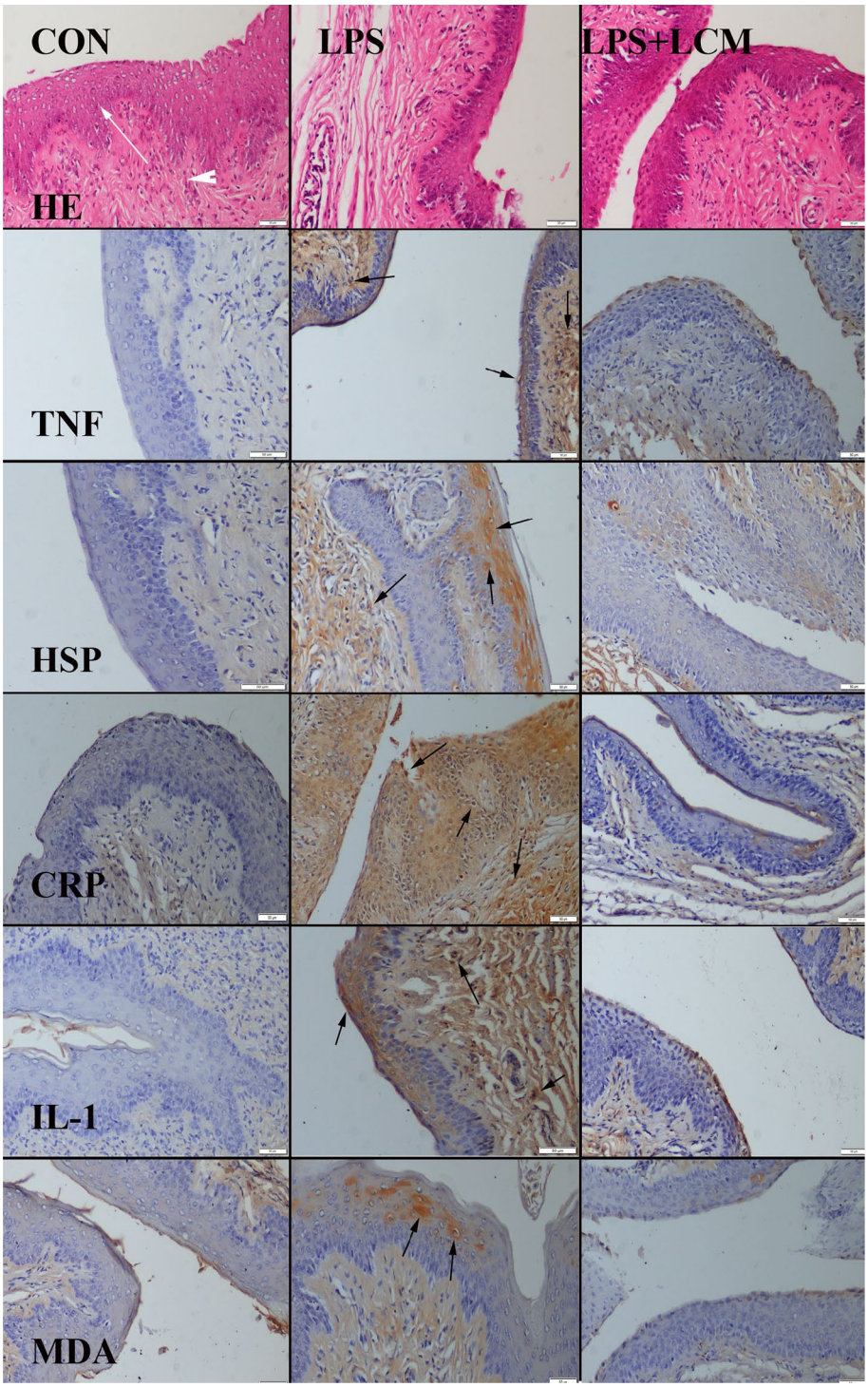

Fig. 5 Vaginal histopathology: normal vaginal epithelium (white arrow) and propria mucosae (white arrow head) in control and LCM-treated group, hyperemia in LPS group, hematoxylin and eosin. Second row; TNF-α, third row: HSP-70, fourth row: CRP, fifth row: IL-1 and sixth row: MDA immunoreaction of the vagina: negative expression in control group; increased immunoreaction in LPS group (arrows), markedly decreased immunoreaction in LCM-treated group, streptavidin–biotin peroxidase method, bars = 50 µ

Likewise, Bitzer-Quintero et al. demonstrated that LPS induced twofold increase in lipid peroxidation levels (MDA and 4-HDA concentrations) showing oxidant and antioxidant status in rats lungs when compared to the control group. In that study, the elevation of MDA levels was prevented by tryptophan and melatonin due to their antioxidant activity [17]. In our study, we detected that lipid peroxidation marker (MDA) values caused by LPS-induced sepsis which resulted from LPS-induced sepsis showed "4.5–11.2" fold increase. LCM ameliorated MDA levels to nearly as low as the ones of the control group.

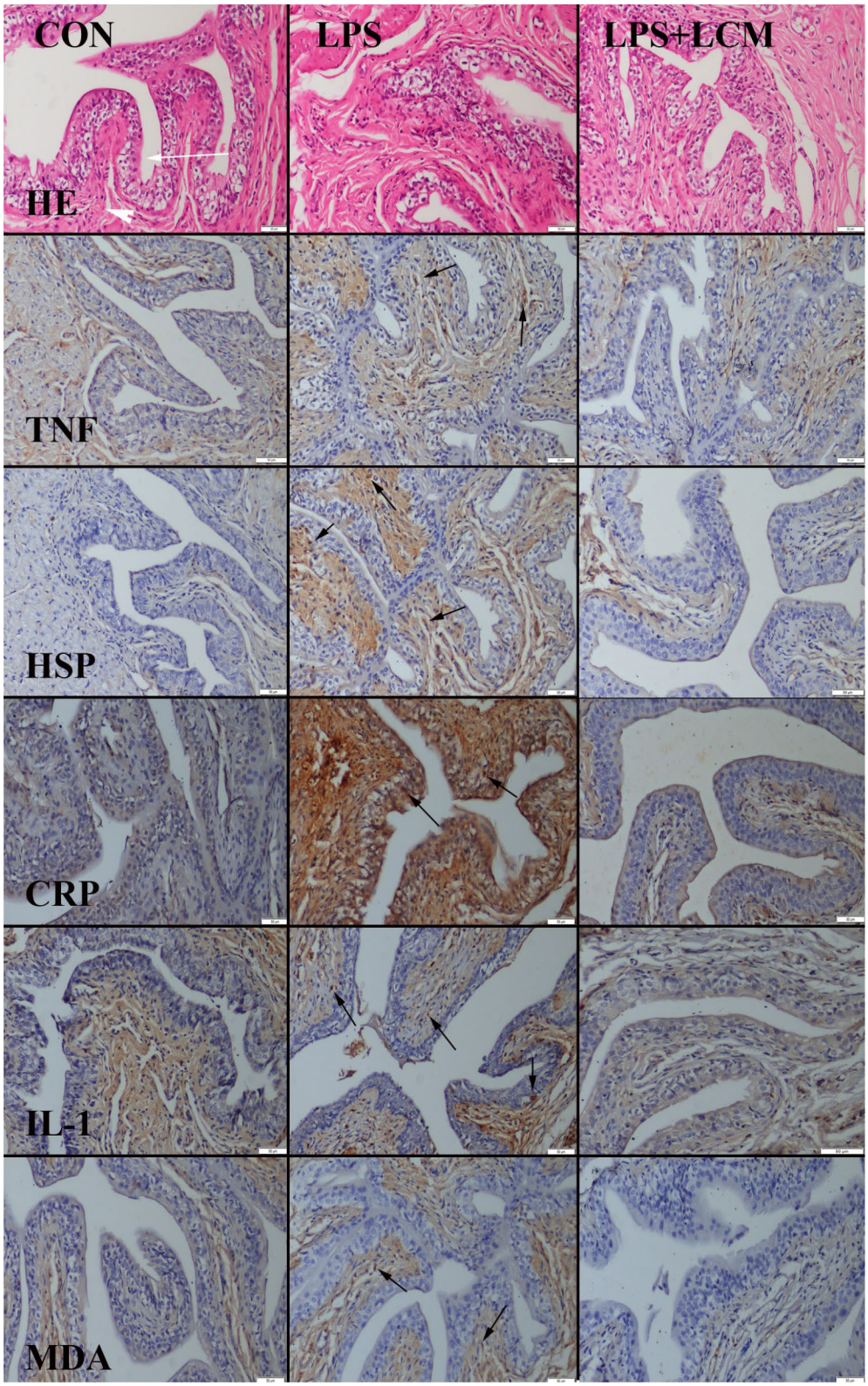

Fig. 6 Urinary bladder histopathology: normal urinary bladder epithelium (white arrow) and propria mucosae (white arrow head) in control, LPS and LCM groups, hematoxylin and eosin. Urinary bladder immunohistochemistry: second row; TNF-α, third row: HSP-70, fourth row: CRP, fifth row: IL-1 and sixth row: MDA immunoreaction of the urinary bladder: slight expression in control group; slight increased immunoreaction in LPS group (arrows), decreased immunoreaction in LCM-treated group, streptavidin–biotin peroxidase method, bars = 50 μ

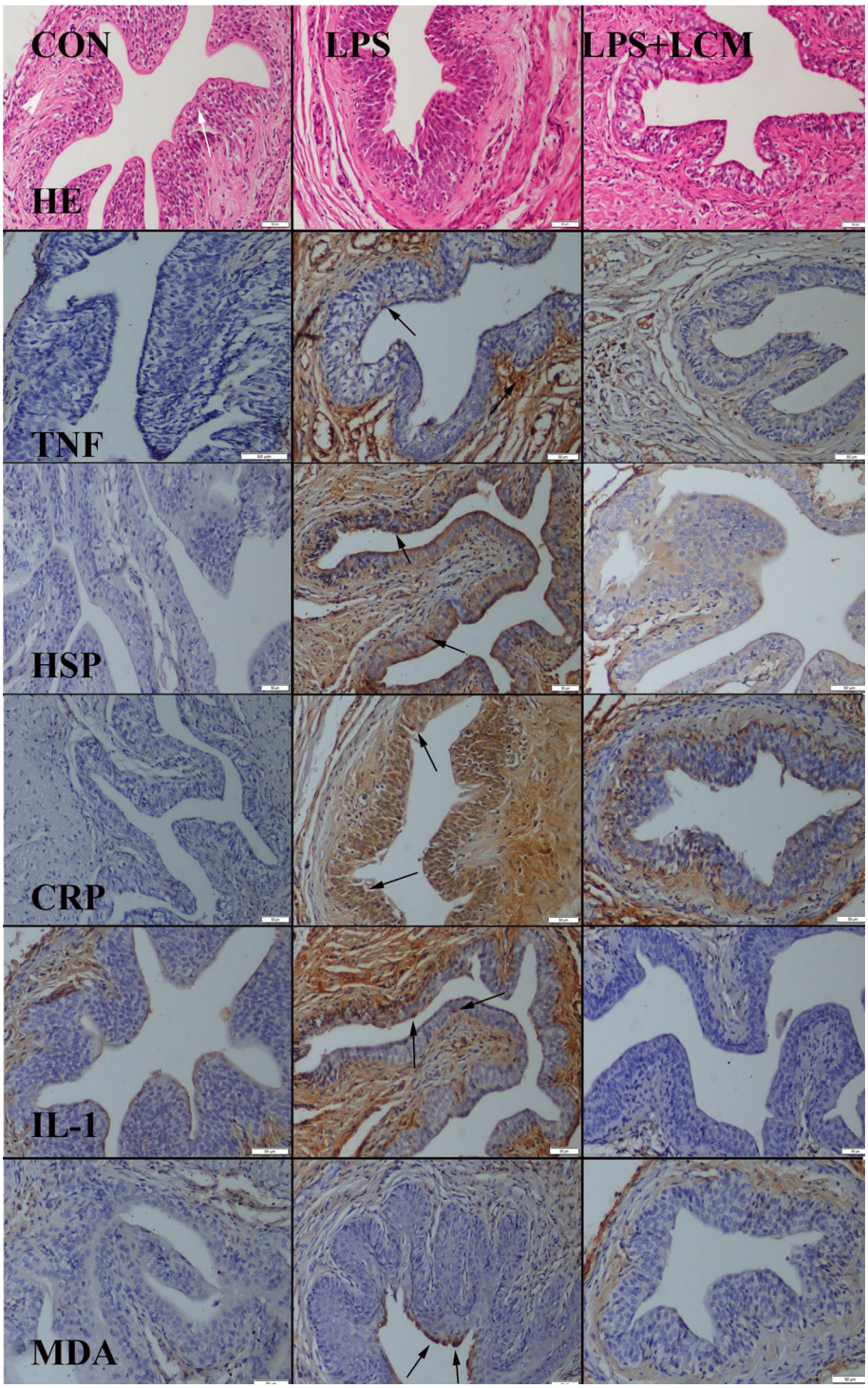

Fig. 7 Urethral histopathology: normal urethral epithelial layer (white arrow) and propria mucosae (white arrow head) in control, LPS and LCM groups, hematoxylin and eosin. Urethral immunohistochemistry: second row; TNF-α, third row: HSP-70, fourth row: CRP, fifth row: IL-1 and sixth row: MDA immunoreaction of the urethra: negative expression in control group; increased immunoreaction in LPS group (arrows), decreased immunoreaction in LCM-treated group, streptavidin–biotin peroxidase method, bars = 50 µ

The amelioration rate was statistically significant in all groups, but slightly reduced in ovarian and vaginal tissues.

Decrease of CRP levels in any pathological condition caused by any treatment means a higher survival rate and improvement. Ersoy et al. demonstrated that an increase in serum CRP levels induced by the experimental rat sepsis was prevented in melatonin-treated group due to its potential antioxidant and potent free radical scavenging effects [15]. Similarly, El-Awady et al. demonstrated the 444% increase in the levels of CRP in sepsis group and its further regression by AGM (up to 178%) [8]. Likewise, in our study, we demonstrated that positive immunohistochemical staining of CRP following sepsis was ameliorated by LCM to nearly the same rate as that of the

Table 4 Statistical analysis of immunohistochemically positive cell numbers

Markers	Organs	Group 1	Group 2	Group 3	*p* value
TNF-α	Ovary	1.87 ± 0.83	12.37 ± 1.50	3.37 ± 0.51	Con-LPS < 0.001 Con-LCM < 0.05 LPS-LCM < 0.001
	Fallopian tube	1.50 ± 0.53	16.12 ± 2.35	4.00 ± 1.41	Con-LPS < 0.001 Con-LCM < 0.05 LPS-LCM < 0.001
	Uterus	3.75 ± 0.70	22.37 ± 1.92	8.37 ± 0.91	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Cervix	4.62 ± 1.40	22.37 ± 2.82	5.75 ± 1.28	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Vagina	3.75 ± 0.88	14.75 ± 2.12	6.00 ± 0.75	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Vesica urinaria	3.75 ± 0.70	17.75 ± 1.03	4.75 ± 1.90	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Urethra	3.25 ± 0.88	19.37 ± 1.40	6.25 ± 1.83	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
HSP-70	Ovary	4.12 ± 1.35	11.00 ± 1.60	3.12 ± 1.12	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Fallopian tube	4.62 ± 1.06	18.00 ± 1.85	8.12 ± 0.83	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Uterus	3.37 ± 0.91	15.62 ± 2.13	11.12 ± 1.24	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Cervix	2.87 ± 1.12	9.75 ± 1.28	2.87 ± 0.99	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Vagina	0.70 ± 0.25	7.87 ± 1.12	2.25 ± 1.03	Con-LPS < 0.001 Con-LCM < 0.01 LPS-LCM < 0.001
	Vesica urinaria	3.62 ± 0.74	15.25 ± 2.54	7.00 ± 1.30	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Urethra	4.25 ± 1.28	13.00 ± 2.92	5.62 ± 1.06	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
CRP	Ovary	5.37 ± 1.40	21.62 ± 2.26	8.37 ± 1.30	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Fallopian tube	2.87 ± 1.35	17.50 ± 4.17	5.87 ± 0.83	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Uterus	4.25 ± 1.16	9.75 ± 3.57	6.12 ± 2.29	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.05
	Cervix	2.37 ± 1.18	11.62 ± 1.40	4.87 ± 1.45	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Vagina	2.25 ± 0.88	25.12 ± 3.27	2.87 ± 1.35	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001

Table 4 (continued)

Markers	Organs	Group 1	Group 2	Group 3	*p* value
	Vesica urinaria	2.62 ± 1.06	23.75 ± 2.25	2.62 ± 1.06	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Urethra	4.00 ± 0.92	20.75 ± 1.28	8.75 ± 1.03	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
IL-1β	Ovary	2.37 ± 0.74	12.62 ± 3.37	2.25 ± 0.70	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Fallopian tube	4.25 ± 1.48	18.62 ± 4.95	6.75 ± 1.66	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Uterus	3.62 ± 2.13	32.37 ± 2.13	7.37 ± 2.38	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Cervix	8.00 ± 1.41	17.87 ± 3.22	4.12 ± 1.55	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Vagina	6.50 ± 2.26	16.50 ± 3.33	7.25 ± 1.16	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Vesica urinaria	7.12 ± 0.99	14.87 ± 2.99	6.87 ± 1.24	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Urethra	8.25 ± 4.86	20.12 ± 1.55	6.50 ± 1.41	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
MDA	Ovary	0.87 ± 0.29	9.75 ± 1.38	5.50 ± 1.06	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Fallopian tube	2.75 ± 1.28	14.00 ± 1.85	3.25 ± 0.70	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Uterus	2.62 ± 1.06	24.50 ± 4.84	5.50 ± 1.19	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Cervix	1.75 ± 0.70	8.00 ± 1.92	2.37 ± 0.74	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Vagina	1.50 ± 0.75	12.00 ± 3.02	5.62 ± 2.13	Con-LPS < 0.001 Con-LCM < 0.001 LPS-LCM < 0.001
	Vesica urinaria	1.00 ± 0.75	11.00 ± 2.07	2.62 ± 1.59	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001
	Urethra	1.62 ± 0.74	10.00 ± 4.89	2.62 ± 1.59	Con-LPS < 0.001 Con-LCM > 0.05 LPS-LCM < 0.001

Values presented as mean ± SE, one-way ANOVA Bonferroni test

Con-LPS: Group 1 vs Group 2; Con-LCM: Group 1 vs Group 3; LPS-LCM: Group 2 vs Group 3

control group in ovary, cervix and urethra, the values were also statistically significant in both GroupI-GroupIII and GroupII-GroupIII. According to our study, the decrease in CRP immunohistochemical staining in LCM-treated group confirms once again the potential anti-inflammatory effect of LCM in LPS-induced rat sepsis model. Therefore, in the current study, we proved that LCM shows the ameliorative effects on urogenital area in sepsis due to its anti-inflammatory, protective and antioxidative effects.

In another LCM study on sepsis [Cecal Ligation and Puncture (CLP)-induced male rat model], Solmaz et al. revealed LCM's ameliorative effects on Critical Illness Neuropathy (CIN) evolving from the severe systemic response syndrome such as sepsis. In this study, LCM's anti-lipid peroxidative and anti-inflammatory effects were evaluated via plasma levels of TNF-α, CRP, MDA and white blood cells (WBC). These levels were increased in sepsis group and decreased in LCM-treated group [18]. Thus, that study is a unique study in the literature evaluating the effects of LCM on polyneuropathy secondary to sepsis. Furthermore, they detected that when CLP + LCM 20 mg/kg and CLP + LCM 40 mg/kg groups were compared, plasma levels of TNF-α were lower in CLP + LCM 40 mg/kg group. For this reason, the LCM dose used in our study, which is LPS + LCM 40 mg/kg, is a good choice, and our preference is confirmed by the literature. Likewise, in our study, we evaluated urogenital system, and the protective effects of LCM via immunohistochemical analysis of TNF-α, CRP, MDA, HSP-70 and IL-1β levels in urogenital tissues. Unlike that research, we studied our markers only immunohistochemically, and in all urogenital tissues. Thus, we showed that all the immunohistochemical markers were ameliorated in LCM-treated group, the same as in the above-mentioned investigation.

In another similar study, Zeng et al. revealed that the administration of polydatin has a therapeutic effect on MODS in septic rats kidney, liver, and lung. They studied TNF-α, IL-1β (and IL-6 levels) as proinflammatory cytokine, apoptosis and oxidative stress markers in serum as in our study. Afterwards, they suggested that the other organs should be investigated as well [20]. On this basis, we can say that the harmful effects of sepsis on urogynecological tissues and ameliorative effects of LCM on sepsis were initially and comprehensively investigated in our study. Lu et al. revealed that serum levels of TNF-α, CRP, and IL-6 were markedly decreased after the Glucocorticoids (GCs) treatment against severe inflammation in experimental rat sepsis model [21]. These results are similar to our study in a way that we revealed that LCM plays an important role in the inflammatory response created by sepsis.

HSP-70 has many useful features such as endogenous cytoprotective, antiapoptotic, increasing anti-inflammatory cytokines, increasing the production of antioxidants, with organ protective effects, as well as decreasing the rat mortality. Likewise, a variety of animals and cellular models, confirmed the existence of a relationship between HSP-70 and sepsis, but the present day clinical observations have failed to establish a direct and clear correlation between HSP-70 modulation and protection against septic shock [22, 23].

Nevertheless, when our results are compared with those in the literature in terms of HSP family, Shen et al. suggested that Raloxifene reduces the severity of sepsis in septic ovariectomized rats due to its anti-inflammatory and antiapoptotic action. They also revealed that the expression of HSP-70 levels was significantly high in LPS-induced sepsis group [7]. Ilgun et al. revealed that HSP-70 levels in serum and liver tissues were significantly increased in sepsis group at the 6th hour and were ameliorated by Glutathione (GSH). This amelioration by GSH is similar to the effects of LCM in our study [24]. In our research, animals were sacrificed 6 h after the last LPS administration. In another study, Gelain et al. demonstrated that HSP-70 serum levels were higher in sepsis non-survivors. Moreover, HSP-70 serum levels were found lower in sepsis survivors in this study [25]. Likewise, in our study we also observed that HSP-70 expression was increased in septic rats and significantly lower in LCM-treated group. These results may indicate that sepsis survival rate will higher for any patients treated with LCM. Eventually, all these results are in agreement with our results. HSP-70 levels were found significantly low in group III compared to group II in all the examined tissues. We observed that HSP-70 levels were reduced particularly in ovarian, uterine cervix, urinary bladder, and urethral tissues similarly to group I. According to these results, we can suggest that LCM has a strong protective effect.

To sum up, our present data indicate that enhanced proinflammatory cytokines secondary to LPS- induced sepsis such as TNF-α, HSP-70, CRP, IL-1β, and MDA were ameliorated by LCM. Our detailed results revealed that LCM pre-treatment may have a protective and ameliorative effect on female urogenital tissues in the current experimental sepsis model via the inhibition of oxidative stress, reduction of proinflammatory cytokine response, and attenuation of apoptosis. In our opinion, further wide ranging, prospective, in vivo human studies, and randomized controlled clinical trials are needed to elucidate and verify the therapeutic effect of LCM on urogenital tissue damage resulting from sepsis.

Conclusion

The ameliorative effects of LCM on urogenital tissues in the experimental murine sepsis model were associated with oxidative stress, apoptosis, as well as reduced systemic inflammatory responses including the suppression of infiltration of inflammatory cells which produced cytokines, For this reason, LCM may be a potential protective or therapeutic agent against sepsis.

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Author's contribution GI contributed to the literature search, project development, manuscript writing/editing, data collection and interpretation, experimental rat study, rat scarification and revision of the article; SM contributed to the biochemical analysis, statistical analysis, and rat sacrification; OO contributed to the histopathological and immunohistochemical analyses, management data analysis, and statistical analysis.

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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