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ORIGINAL ARTICLE

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Dapagliflozin prevents reproductive damage caused by acute systemic inflammation through antioxidant, anti-inflammatory, and antiapoptotic mechanisms

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Abstract

Dapagliflozin (DPG) is a sodium-glucose cotransporter-2 (SGLT2) inhibitor that has been suggested to possess anti-inflammatory properties in diabetes. The aim of this study is to evaluate the role of DPG administration in preventing lipopolysaccharide (LPS)-induced damage in the female genital system. Thirty-two female Wistar Albino rats were randomly allocated into four groups: control group, LPS group, LPS + DPG group and DPG group. At the end of the experimental phase, ovary, fallopian tube and uterus tissues were collected for histopathological, immunohistochemical, genetic and biochemical analyses. The findings showed that LPS caused histopathological changes characterized by marked hyperaemia, mild to moderate haemorrhage, oedema and neutrophil leucocyte infiltrations and degenerative and necrotic changes in the female genital tract. In addition, it decreased total antioxidant status (TAS), increased total oxidant status (TOS) and oxidative stress index (OSI) levels. LPS also increased the expressions of Cas-3, G-CSF and IL-1β in the ovary, fallopian tubes and uterus immunohistochemically. While Claudin-1 expression decreased, NLRP3 and AQP4 gene expressions increased due to LPS. However, DPG treatment prevented all these changes. The results of this study indicate that, DPG can be used to prevent LPS-induced lesions in the female reproductive system.

KEYWORDS
dapagliflozin, female reproductive system, lipopolysaccharide

Plain English Summary

This study investigates the protective effects of dapagliflozin (DPG), a diabetes medication, against inflammation caused by lipopolysaccharide (LPS) in the female reproductive system. Female rats were divided into four groups and treated with LPS and/or DPG. The results showed that LPS caused significant damage to the ovaries, fallopian tubes and uterus, including inflammation, oxidative stress and changes in protein and gene expression. However, DPG treatment prevented these harmful effects, suggesting that DPG may protect against inflammation-related damage in the female reproductive system.

1 | INTRODUCTION

Gram-negative bacteria protect themselves with two phospholipid membranes. The outer membrane contains a glucosamine-based phospholipid called lipopolysaccharide (LPS), a well-known endotoxin that exerts toxic effects on the host when released from lysed bacteria is the main cause of sepsis or endotoxemia.[1,2] Endotoxemia or systemic inflammation can be triggered by various factors, such as diabetes, obesity, environmental chemical exposure, abiotic stressors, and bacterial infections, which can affect morbidity and mortality by impacting multiple tissues through the increased formation of pro-oxidant and pro-inflammatory substances in the blood..[3,4] These substances, which reach the genital system through the blood, are known to trigger damage in different genital tissues, causing dysfunction and decreasing the fertility rate. Circumstances that negatively impact intestinal barrier integrity can lead to endotoxemia. Studies have shown that LPS can have adverse effects on folliculogenesis, puberty onset, oestrus behaviour, ovulation, meiotic competence, luteal function and ovarian steroidogenesis.[5,6] A positive correlation has been identified between the increase in blood LPS levels and cytokine levels in ovarian follicles, suggesting that this condition may cause damage to oocytes and ovaries.[7]

In the literature, antioxidant, anti-inflammatory and antiapoptotic agents have been tried to prevent this situation.[8–10] It is known that some cellular systems such as NLR family pyrin domain-containing 3 (NLRP3) pathway are activated in inflammation occurring in tissues, and the resulting inflammation causes significant histopathological changes in tissues.[11–13] It has been reported that decreased levels of Claudin-1, an important indicator of intercellular connections, are associated with increased damage pictures and increased levels of aquaporin-4 (AQP4), which contributes to the excretion of cellular wastes.[14–17]

Dapagliflozin (DPG), an inhibitor of sodium-glucose cotransporter-2 (SGLT-2), which inhibits glucose transport through the intestines, is a widely used antidiabetic agent. In the treatment of diabetes mellitus, a chronic disease, studies have been conducted on its additional protective effects at the tissue level.[18,19] It has been reported that the increased doses and long-term use of DPG, due to its antidiabetic effect of increasing glycosuria, increase the incidence of genitourinary system infections.[20–22] DPG's antihyperglycaemic, cardioprotective and renoprotective properties make it stand out in diabetes treatment.[23]

The high prevalence of diabetes and its potential to alter critical elements of sepsis pathophysiology have shown that it is an important comorbid condition in this disease. Numerous preclinical and clinical studies have been conducted to discuss the influence of diabetes on sepsis pathophysiology, susceptibility and clinical outcomes.[24–26] Inflammation and insulin resistance driven by chronic and stress-induced hyperglycaemia, along with obesity and dyslipidaemia associated with type 2 diabetes (T2D), are among the various metabolic abnormalities that further worsen the host response against infections.[27]

The aim of the present study was to investigate the preventive effect of DPG on the reduction of ovarian, fallopian and uterine tissue damage secondary to LPS-induced systemic inflammation and the role of Claudin-1, NLRP3 and AQP4 expressions in this effect.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[28] The procedures conducted on rats were subjected to review and approval by the Animal Experiments Local Ethics Committee of Suleyman Demirel University (Ethic No: 28.03.2024-04/277). The experiment adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines. Furthermore, support for the study was provided by the Suleyman Demirel University Scientific Research Project Unit under the project number TSG-2023-9010.

2.2 | Chemicals

LPS was obtained from Sigma Aldrich (L2630-100 mg), USA. DPG (Forziga 10 mg), was obtained from Astra Zeneca, Türkiye. To induce sedation and anaesthesia applications, Xylazine (Xylazinbio %2, Bioveta, Czech Republic) and Ketamine (Ketalar, Pfizer, Türkiye) were used.

2.3 | Animals

Using GPower 3.1.9.7 software, a study was planned on four groups, each consisting of eight rats (total sample size of 32), considering the relevant parameters ($\alpha = 0.05$, $1\text{-}\beta = 0.90$, effect size = 0.32). Animals were housed in Euro type-2 cages using wood shavings as litter and were maintained in an environment in which the temperature between 22 and 23°C, with a 12-h light–dark cycle. The 32 female Wistar Albino rats weighing 300–350 g were utilized with libitum feeding regime. Four groups assigned for the experimental model, each containing eight rats and randomly distributed:

1. Control group: 0.5 cc of saline was administered intraperitoneally (i.p.) administered after 1 cc of isotonic saline via oral gavage (p.o.) for five consecutive days.
2. LPS group: LPS, dissolved in saline, was administered i.p. to rats at a dose of 5 mg/kg. This was done 6 h prior to sacrifice, following 5 days of oral administration of 1 cc of isotonic saline.[29]
3. LPS + DPG group: 5 mg/kg i.p. LPS was administered after 5 days application of 10 mg/kg DPG p.o.[30]
4. DPG group: 0.5 cc of saline was administered i.p. after 10 mg/kg DPG p.o. for 5 days.

Animals were sacrificed under 80 mg/kg Ketamine (Ketalar, Pfizer, Türkiye) and 10 mg/kg Xylazine (Xylazinbio %2, Bioveta, Czech Republic) anaesthesia following 6 h after LPS application. After the abdominal incision, surgical exsanguination was performed, and ovarian, fallopian and uterine tissues were removed. Half of the tissues were kept in 10% buffered formalin solution for histopathologic analysis. Remaining of fallopian and uterine tissues were put into −20°C for oxidative stress parameters by biochemical analysis, and ovarian tissues were put into −80°C for Claudin-1, NLRP3 and AQP4 by genetic analysis.

2.4 | Biochemical analysis

To initiate the experiment, tissues were diluted in phosphate-buffered saline (10 mM sodium phosphate) with a fivefold weight/volume ratio, adjusting the pH to 7.4. Subsequently, the tissues underwent homogenization using a tissue homogenizer (IKA Ultra Turrax T25, Janke & Kunkel, Staufen, Germany). Following homogenization, samples were centrifuged at 2000 rpm for 20 min at +4°C using a Nuve NF 1200R centrifuge (Ankara, Türkiye). The supernatant obtained after centrifugation was utilized to measure the concentrations of tissue total antioxidant status (TAS) and total oxidant status (TOS). An automated biochemistry analyser (Beckman Coulter AU 5800, Brea, CA, USA) and colorimetric methods developed by Erel were employed for these assays.[31,32] TOS results were expressed as μmol H2O2 Equiv/g, while TAS results were reported as mmol Trolox Equiv/g. The oxidative stress index (OSI) was calculated by dividing TOS levels by TAS levels, denoted as TOS/TAS/10.[33]

2.5 | Histopathological examinations

Genital system tissues, including the ovarium, fallopian tube and uterus, were grossly examined during the necropsy. Following that, tissue samples were harvested and preserved in neutral buffered formalin (10%). Leica ASP300S (Leica Microsystems, Wetzlar, Germany), a fully automatic tissue processor, was used to process tissue samples for routine tissue processing method. After the samples were embedded in paraffin wax, a Leica RM2155 rotary microtome (Leica Microsystems, Wetzlar, Germany) was used to cut the samples into 5-μm sections. Haematoxylin–eosin (HE) staining was applied to all the tissues, and a specialized histopathologist from a different university, who was not informed of the groups, evaluated the samples under a light microscope.

The ovarium, fallopian tube and uterine histopathological lesions were graded using a semiquantitative scoring method. Evaluations were conducted on parameters including hyperaemia, oedema, haemorrhage, infiltrations of inflammatory cells, degeneration and epithelial loss. Scores ranging from 0 to 3 were allocated to descriptions according to the extent of the lesions.

2.6 | Immunohistochemical examinations

A streptavidin-biotin complex technique was used to immunostain the selected sections from the organs of the genital system with active caspase-3 [Anti-Caspase-3 antibody [EPR18297] (ab184787)], granulocyte colony-stimulating factor [Recombinant Anti-G-CSF antibody [CSF3/3166R] (ab270267)] and IL-1β [Anti-IL-1 beta

antibody [EPR24895-116] (ab315084)]. Abcam (Cambridge, UK) provided the primary and secondary antibodies that were purchased. After the sections were treated with the primary antibodies for 60 min, biotinylated secondary antibodies and streptavidin–alkaline phosphatase conjugate were used for immunohistochemistry. The secondary antibody utilized in the Mouse and Rabbit Specific HRP/DAB IHC Detection Kit was Micropolymer (ab236466). Using diaminobenzidine (DAB) as the chromogen, the antigens were shown. For every marker, there were positive controls. We skipped the primary antiserum stage for the negative controls.

Every evaluation was administered in a blinded fashion. At a magnification of ×40, 100 cells were counted from each group to determine the percentage of positive cells for each marker in each area. The ImageJ programme (version 1.48, National Institutes of Health, Bethesda, MD) was used to analyse the images. A statistical analysis was performed on the image analyser's data. The Database Manual CellSens Life Science Imaging Software (Olympus Co., Tokyo, Japan) was utilized to conduct morphometric studies.

2.7 | Reverse transcription-polymerase chain reaction (RT-qPCR)

Using the manufacturer's protocol, RNA was isolated from homogenized tissues with the GeneAll RiboEx (TM) RNA Isolation Kit (GeneAll Biotechnology, Seoul, Korea). The quantity and purity of the obtained RNAs were measured using the BioSpec-nano nanodrop device (Shimadzu Ltd. Kyoto, Japan) device; 1 μg RNA was used for cDNA synthesis. cDNA synthesis, A.B.T. ™ cDNA Synthesis Kit (Atlas Biotechnology, Türkiye) was carried out in a thermal cycler according to the protocol. Primer designs were made by detecting specific mRNA sequences and testing possible primer sequences using the NCBI website. The sequences of the primer sequences used are shown in Table 1. Expression levels of genes were measured in a Biorad CFX96 (California/USA) real-time PCR instrument using 2X SYBR green master mix (Nepenthe/Türkiye). In the study, the GAPDH gene was used as a housekeeping gene. The reaction mixture was prepared according to the manufacturer's protocol to a final volume of 20 μL. The resulting reaction mixture was placed in a real-time qPCR device determined according to the kit manufacturer's protocol, and each sample was studied in three replications. PCR conditions, initial denaturation 94°C 10 min 1 cycle, denaturation 95°C 15 s and annealing/extension 55°C 30 s were applied as 40 cycles. Relative mRNA levels were calculated by applying the $2^{-\Delta\Delta Ct}$ formula to the normalized results.

2.8 | Statistical analysis

Initially, the Shapiro–Wilk method was employed to assess the normality of the data distribution. ANOVA was employed as a means of comparing the groups since the data showed a normal distribution ($p > 0.05$). For the comparison between the groups, one-way ANOVA post hoc Tukey test with Graphpad prism programme have been used for statistical analysis, and $p < 0.05$ was considered significant.

3 | RESULTS

3.1 | Biochemical results

Biochemical analyses were performed; TAS values were significantly decreased in the LPS group compared to the control group ($p = 0.011$), and a nonsignificant increase

TABLE 1 Primary sequences, product size and accession numbers of genes.

Genes	Primary sequence	Product size	Accession number
GAPDH (HouseKeeping)	F: AGTGCCAGCCTCGTCTCATA	248 bp	NM_017008.4
	R: GATGGTGATGGGTTTCCCGT		
Claudin 1	F: ACTGTGGATGTCCTGCGTTT	127 bp	NM_031699.3
	R: CCCCAGCAGGATGCCAATTA		
NLRP3	F: TCTCTGCATGCCGTATCTGG	295 bp	NM_001191642.1
	R: ACGGCGTTAGCAGAAATCCA		
AQP4	F: TTGGACCAATCATAGGCGC	212 bp	XM_039096587.1
	R: GTCAATGTCGATCACATGC		

Abbreviations: AQP4, aquaporin 4; F, forward; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NLRP3, NLR family pyrin domain-containing 3; R, reverse.

was found in the LPS + DPG group compared to the LPS group ($p < 0.05$). TOS and OSI values were significantly higher in the LPS group compared to the control group ($p = 0.004$ and $p < 0.001$, respectively) and the DPG group ($p < 0.001$ for both). On the other hand, TOS and OSI values were significantly decreased in the LPS + DPG group compared to the LPS group ($p = 0.004$ and $p < 0.001$, respectively) (Figure 1).

3.2 | Histopathological analyses

Gross inspection showed that the LPS group had mild to moderate hyperaemia, while the control, DPG and LPS-DPG groups were normal. Animals in different oestrous cycles were detected in all groups. Evaluations were primarily made between groups of animals in the same phase. Findings that were not observed in the control group, despite being in the same oestrous phase, were interpreted as the effects of LPS. Animals in the dioestrus phase were selected for the photographs. Histopathological analysis of the genital system's investigated organs in the control and DPG groups revealed no pathology results. In the LPS group, there was significant hyperaemia mild to moderate haemorrhage, oedema and neutrophil leucocyte infiltrations. Furthermore, endometrial damage and inflammatory cell infiltrations in the uterus and cilia loss in fallopian tube were also observed in this group. DPG therapy prevented all pathological findings in every organ (Figure 2).

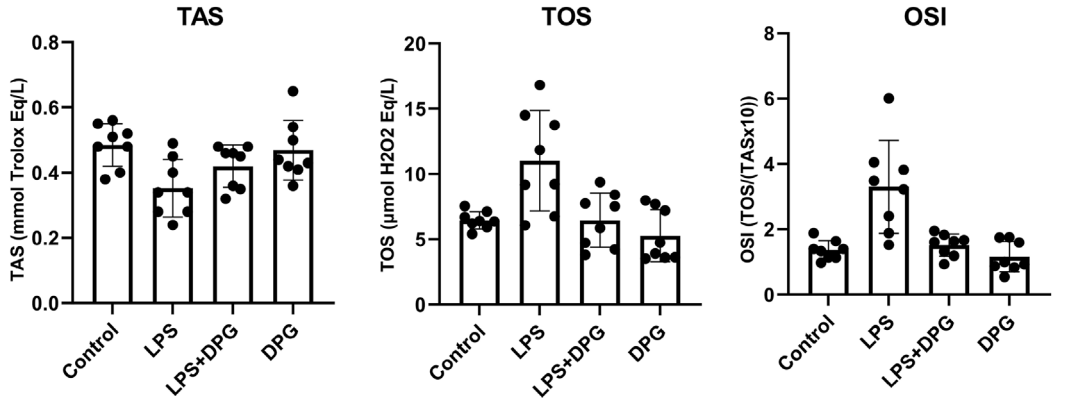
3.3 | Immunohistochemical analyses

Immunohistochemical analysis showed that negative to slight expressions in control and DPG groups. The LPS group's ovaries, fallopian tubes and uterus all had marked expressions of Cas-3, G-CSF and IL-1β. The results of the immunohistochemistry and histopathology were significantly improved by DPG. Graphics represents the findings of the statistical analysis of the immunohistochemically positive cell counts (Figures 3–5).

When there was a statistically significant increase in all expressions in ovarian tissues treated with LPS, luteal and mesenchymal cells showed the most frequent expressions in the ovarian tissue, and comparable expressions were noted in the tissues of the uterus and fallopian tubes. DPG therapy reduced all markers' expressions increased in response to LPS. The expression of all markers was restricted to the cytoplasm, and it was present in both mesenchymal and epithelial cells. No or very little expressions were shown by either the control or DPG groups, and there was no statistically significant difference between them.

3.4 | Genetic results

Claudin-1 levels were significantly lower in the LPS group than in the control and DPG groups ($p < 0.001$ for both), while they were significantly higher in the LPS + DPG group than in the LPS group. In addition,



Control vs. LPS	ns	,976
Control vs. LPS+DPG	ns	,366
Control vs. DPG	*	,011
LPS vs. LPS+DPG	ns	,607
LPS vs. DPG	*	,030
LPS+DPG vs. DPG	ns	,333

Control vs. LPS	ns	,766
Control vs. LPS+DPG	ns	>,999
Control vs. DPG	**	,004
LPS vs. LPS+DPG	ns	,768
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	**	,004

Control vs. LPS	ns	,954
Control vs. LPS+DPG	ns	,979
Control vs. DPG	***	<,001
LPS vs. LPS+DPG	ns	,800
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	***	<,001

FIGURE 1 Oxidative stress parameters of fallopian and uterine tissues. DPG, dapagliflozin; LPS, lipopolysaccharide; OSI, oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status. Values are presented as means ± standard deviation. One-way ANOVA test was used. *$p < 0.05$, **$p \leq 0.01$ ***$p \leq 0.001$.

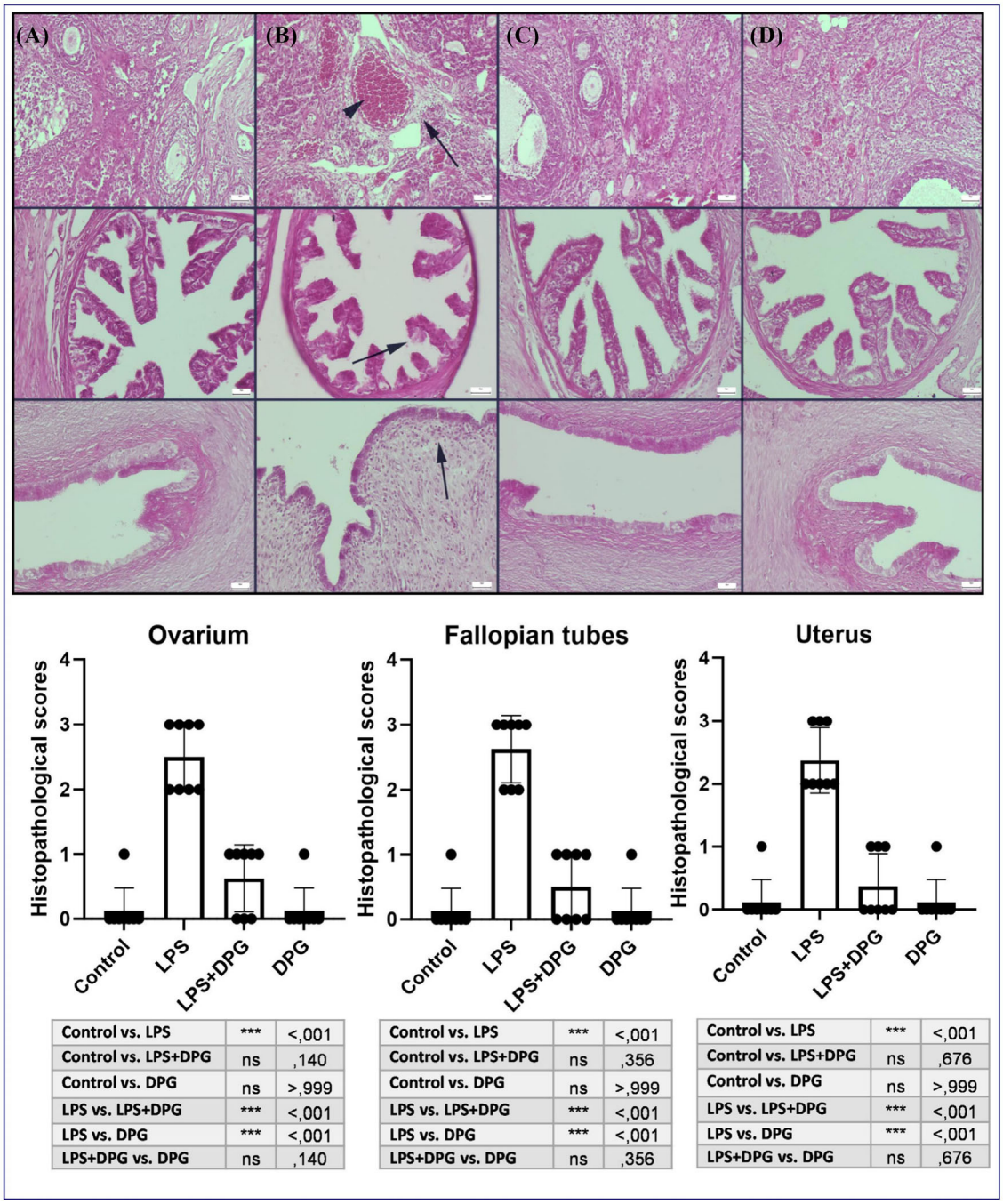


FIGURE 2 Representative histopathological findings of ovarium (upper row), fallopian tubes (medium row) and uterus (below row) between the groups. (A) Normal ovarium, fallopian tube and uterine histology in control group. (B) Marked hyperaemia in ovarium (arrow), cilia loss in fallopian tube (arrow) and epithelial loss, inflammatory cell infiltrations (arrow) in endometrium in the lipopolysaccharide (LPS) group. (C) Decreased pathological findings in the LPS + DPG group, (D) normal tissue architecture in dapagliflozin (DPG) group, HE, scale bars = 50 μm. Values are presented as means ± standard deviation. One-way ANOVA test was used. *$p < 0.05$, **$p \leq 0.01$ ***$p \leq 0.001$.

Claudin-1 expression in the DPG-only group was not statistically significantly different from the control and LPS + DPG groups ($p < 0.05$).

NLRP3 and AQP4 expressions were significantly increased in the LPS group compared to the control and DPG groups, while both parameters were significantly decreased in the LPS + DPG group compared to the LPS group ($p < 0.001$ for all). There was no statistical significance in the DPG-only group compared to the control and treatment groups (Figure 6).

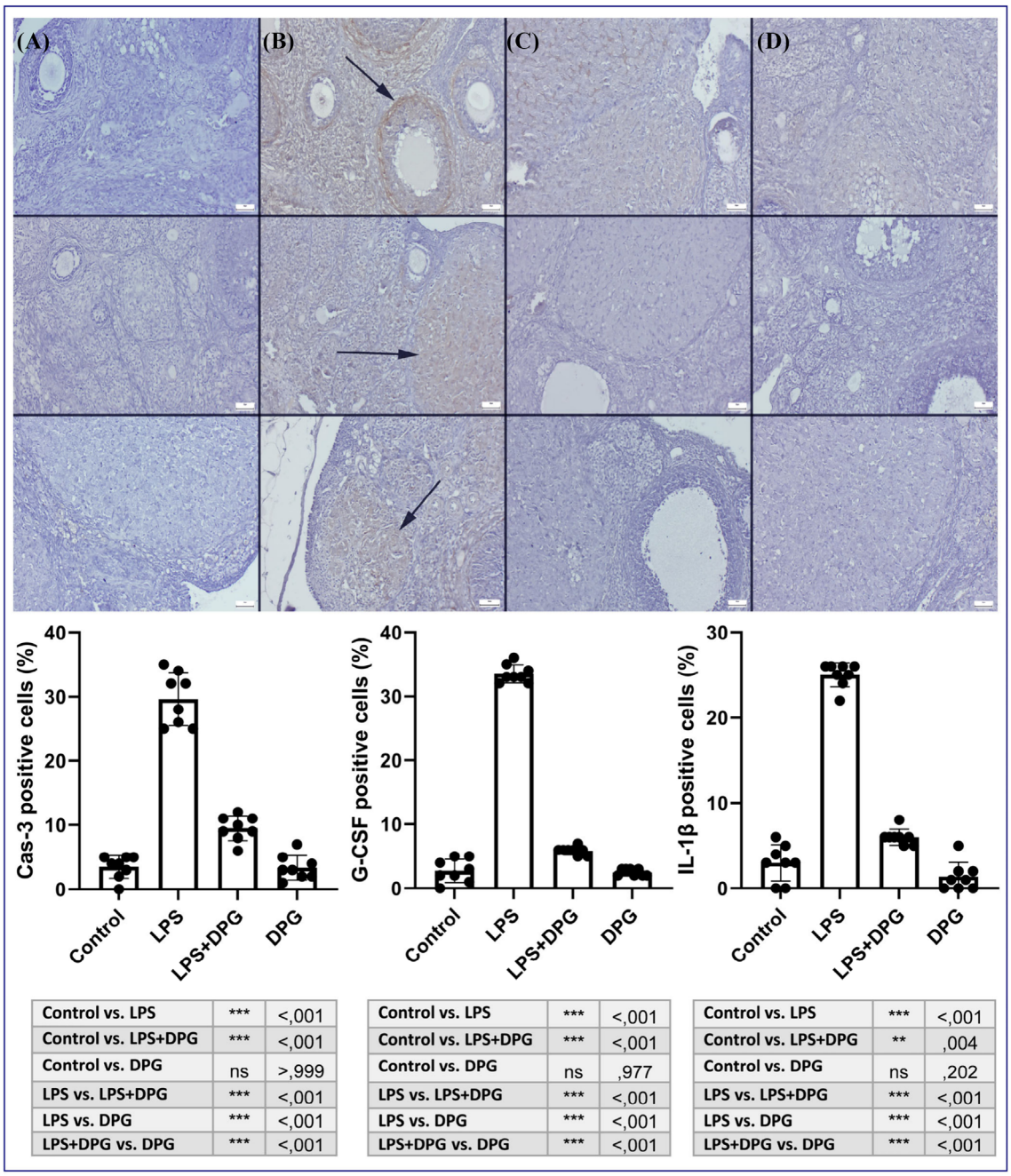
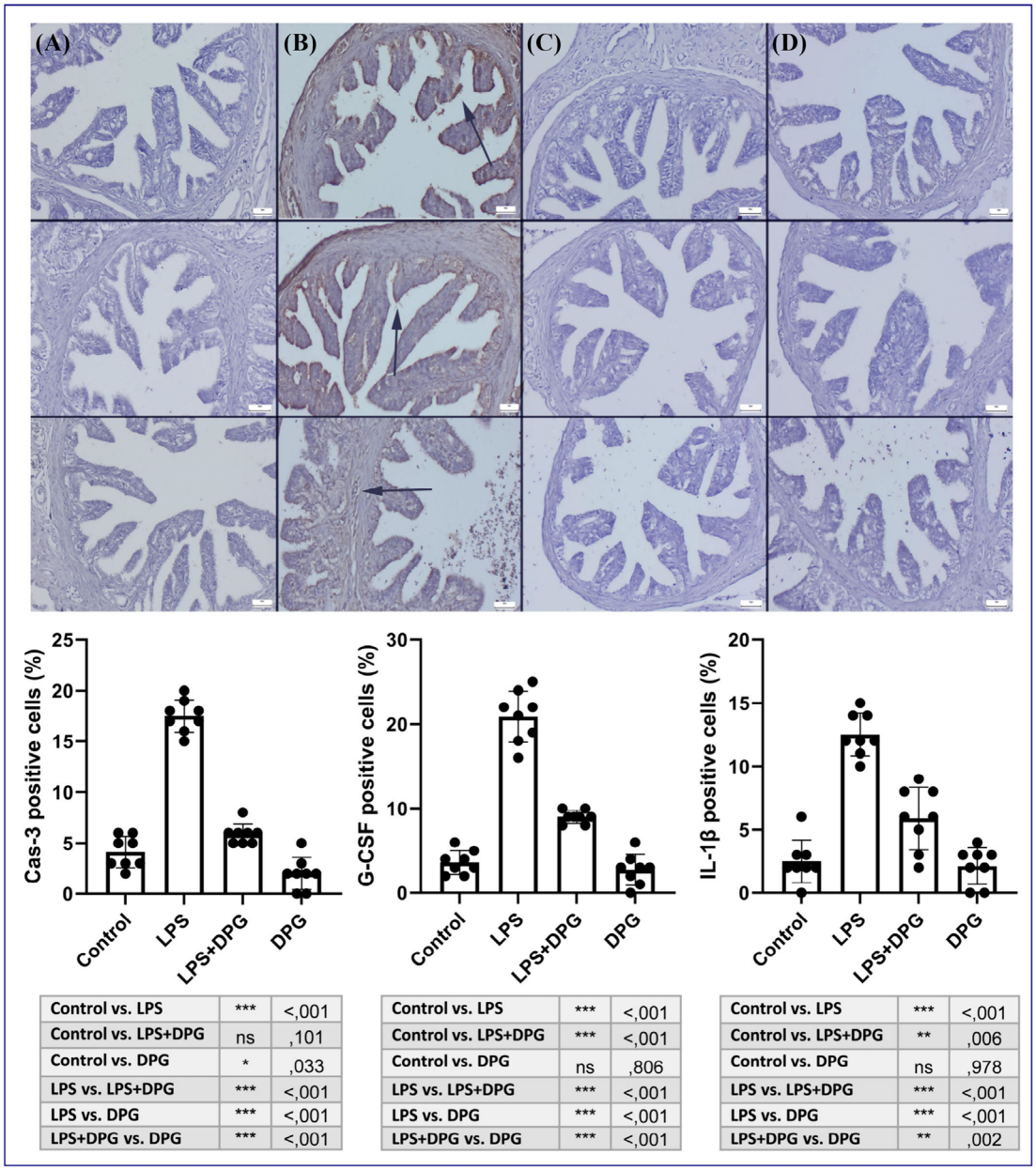


FIGURE 3 Ovarian immunohistochemical Cas-3 (upper row), G-CSF (medium row) and IL-1β (below row) expression and statistical analyses of immunopositive cell percentage between the groups. (A) Negative to slight expressions in the control group, (B) markedly increased expressions (arrows) in the lipopolysaccharide (LPS) group, (C) decreased expression in the LPS + DPG group and (D) negative to very slight expressions, streptavidin biotin peroxidase method, scale bars = 50 μm. Values are presented as means ± standard deviation. One-way ANOVA test was used. *$p < 0.05$, **$p \leq 0.01$ ***$p \leq 0.001$.

4 | DISCUSSION

Due to the increasing prevalence of T2D worldwide, it has become a subject of extensive research. A growing array of antidiabetic medications targeting different organ systems involved in the pathophysiology of T2D has been developed. Among these medications, SGLT-2 inhibitors have recently been added. This group of drugs reduces renal glucose reabsorption, leading to glucosuria, alleviation of hyperglycaemia and modest weight loss, and is associated with a low risk of hypoglycaemia. In addition to these beneficial effects, studies have shown that SGLT-2 inhibitors increase the incidence of genital mycotic infections and, to a lesser extent, urinary tract



Control vs. LPS	***	<,001
Control vs. LPS+DPG	ns	,101
Control vs. DPG	*	,033
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	***	<,001

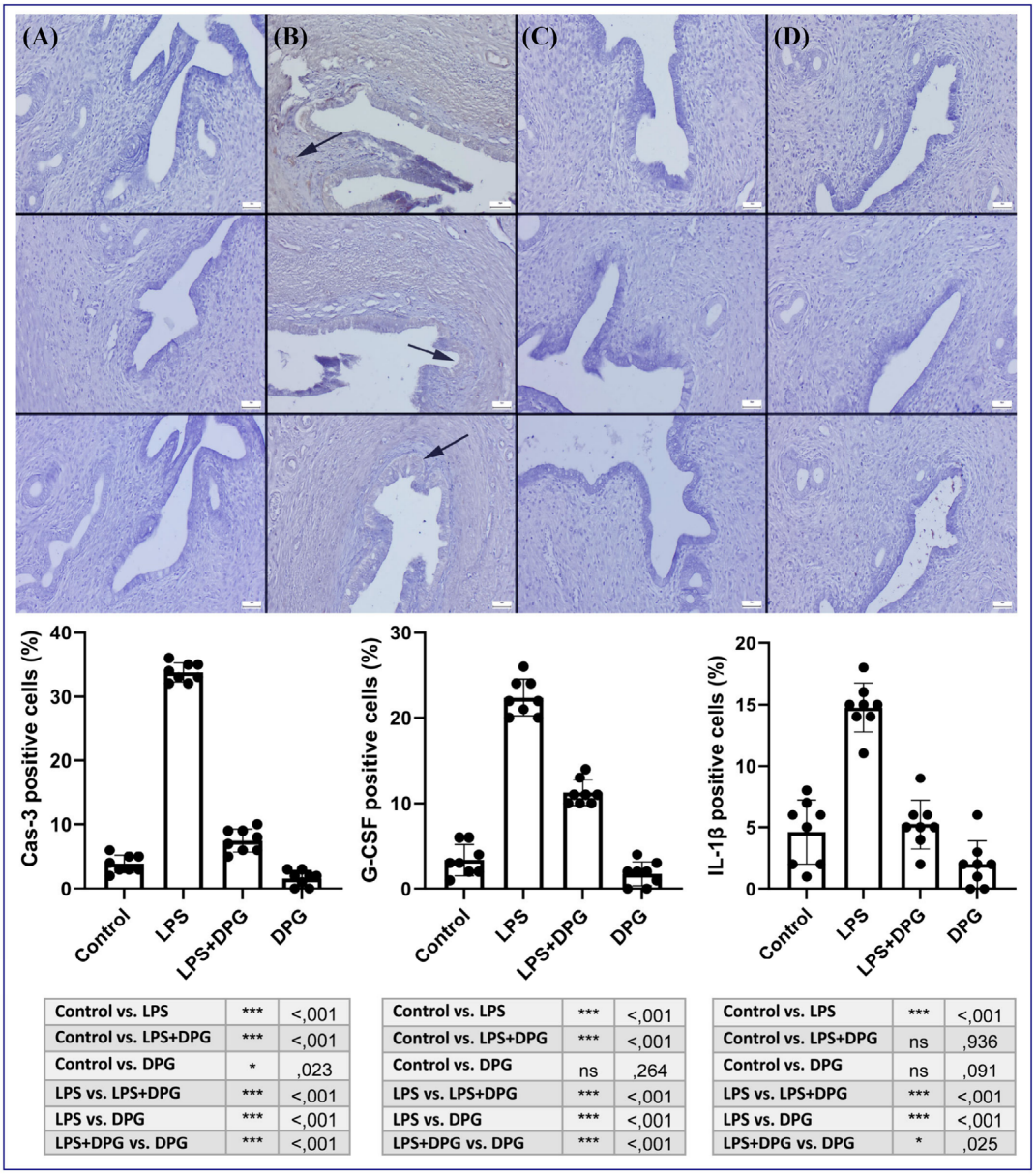
Control vs. LPS	***	<,001
Control vs. LPS+DPG	***	<,001
Control vs. DPG	ns	,806
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	***	<,001

Control vs. LPS	***	<,001
Control vs. LPS+DPG	**	,006
Control vs. DPG	ns	,978
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	**	,002

FIGURE 4 Fallopian tube immunohistochemical Cas-3 (upper row), G-CSF (medium row) and IL-1β (below row) expression and statistical analyses of immunopositive cell percentage between the groups. (A) Negative or very slight expressions in the control group, (B) markedly increased expressions (arrows) in the lipopolysaccharide (LPS) group, (C) decreased expression in the LPS + DPG group and (D) negative to very slight expressions, streptavidin biotin peroxidase method, scale bars = 50 μm. Values are presented as means ± standard deviation. One-way ANOVA test was used. *$p < 0.05$, **$p \leq 0.01$ ***$p \leq 0.001$.

infections, which may limit their long-term use in some patients. However, among this group of drugs, DPG appears to be relatively safer, with these risks being reported as comparatively lower.[20–22,34] This study evaluates the preventive effects of DPG on the detrimental effects of sepsis on the ovaries, fallopian tubes and uterus in a rat model.

In this study, LPS-induced systemic inflammation increased oxidative stress in the fallopian and uterine tissues of the female genital organs. In addition to these organs, significant hyperaemia, mild to moderate haemorrhage, oedema and neutrophil leucocyte infiltrations were detected in all tissues in histopathological and immunohistochemical analyses performed in ovarian tissue. On the other hand, endometrial damage and inflammatory cell infiltrations in the uterus and cilia loss in fallopian tube were observed. Genetic analysis of ovarian tissue showed that Claudin-1 expression decreased, while NLRP3 and AQP4 expression increased. DPG treatment prevented all the adverse effects observed in the tissues.



Control vs. LPS	***	<,001
Control vs. LPS+DPG	***	<,001
Control vs. DPG	*	,023
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	***	<,001

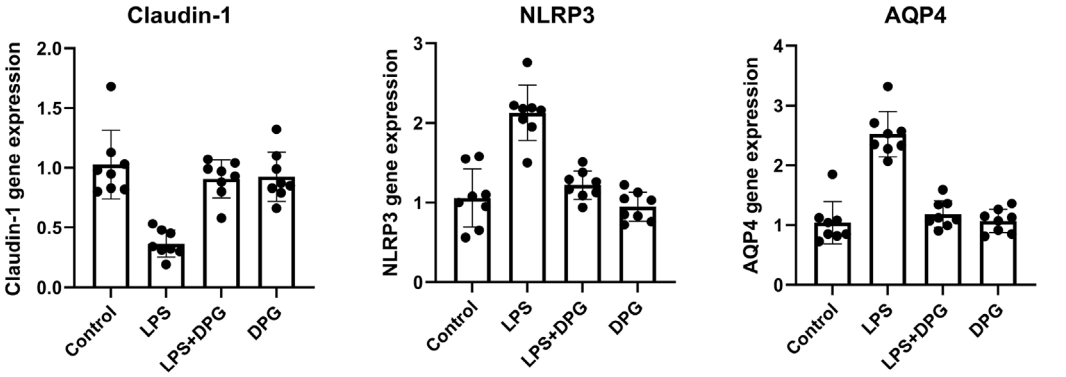
Control vs. LPS	***	<,001
Control vs. LPS+DPG	***	<,001
Control vs. DPG	ns	,264
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	***	<,001

Control vs. LPS	***	<,001
Control vs. LPS+DPG	ns	,936
Control vs. DPG	ns	,091
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	*	,025

FIGURE 5 Uterine immunohistochemical Cas-3 (upper row), G-CSF (medium row) and IL-1β (below row) expression and statistical analyses of immunopositive cell percentage between the groups. (A) Negative to slight expressions in the control group, (B) markedly increased expressions (arrows) in the lipopolysaccharide (LPS) group, (C) decreased expression in the LPS + DPG group and (D) negative to very slight expressions, streptavidin biotin peroxidase method, scale bars = 50 μm. Values are presented as means ± standard deviation. One-way ANOVA test was used. *$p < 0.05$, **$p \leq 0.01$ ***$p \leq 0.001$.

Chronic diseases such as diabetes, which are widely observed, can cause functional disorders in many organs, as well as dysfunction in the female genital system, resulting in clinical findings such as infertility.[35,36] The damage caused by such diseases at the tissue level may involve cellular mechanisms such as inflammation, oxidative stress and apoptosis.[37–39] Therefore, the agents that can be used in the treatment of these diseases are expected to have antioxidant, anti-inflammatory and antiapoptotic properties in addition to their main therapeutic mechanisms. Thanks to these extra properties, it will be a positive approach to reduce the number of drugs that patients will use in a number of different pathological processes, especially during pregnancy, in order to minimize the side effects that may develop due to drugs.

It has been found that proinflammatory and prooxidant molecules in the blood play a role in triggering these mechanisms in tissues. The receptors of oxidant and inflammatory substances on the cell surface can trigger



Control vs. LPS	***	<,001
Control vs. LPS+DPG	ns	,638
Control vs. DPG	ns	,748
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	ns	,998

Control vs. LPS	***	<,001
Control vs. LPS+DPG	ns	,665
Control vs. DPG	ns	,865
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	ns	,242

Control vs. LPS	***	<,001
Control vs. LPS+DPG	ns	,787
Control vs. DPG	ns	,998
LPS vs. LPS+DPG	***	<,001
LPS vs. DPG	***	<,001
LPS+DPG vs. DPG	ns	,869

FIGURE 6 The expression levels of Claudin-1, NLR family pyrin domain-containing 3 (NLRP3) and AQP4 in genital tissues. AQP4, aquaporin 4; DPG, dapagliflozin; LPS, lipopolysaccharide; NLRP3, NLR family pyrin domain-containing 3. Values are presented as means ± standard deviation. One-way ANOVA test was used. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

oxidative stress, inflammation and apoptosis, which is programmed cell death, by creating intracellular signalling. This chaotic damage caused by these mechanisms, which can also stimulate each other, is known to cause organ dysfunctions.[40] It has been proven that oxidative stress, one of the most well-known of these damages, is observed in the fallopian tubes and uterus and may cause inhibition of fertilization as a result of disruption of the physiological environment required for implantation after sperm and ovum union in the tissue.[38,41] In this study, the results of oxidant and antioxidant enzyme activity, which were analysed by spectrophotometric method, showed that systemic inflammation caused oxidative stress in tubal and uterine tissues. DPG, which is used as an antidiabetic and increase glucosuria with its SGLT-2 inhibitor activity, has the advantage of having a tissue protective function with its oxidative stress-reducing effects.

As is known, the damage caused by oxidant substances at the tissue level can activate a number of cellular pathways, often resulting in inflammation and apoptosis.[42] Increased production of reactive oxygen species causes damage to crucial biological components such as nucleic acids, lipids and proteins.[43] Numerous studies have demonstrated that oxidative stress may have a role, albeit to varying degrees, in the development and/or progression of a number of illnesses.[44–46] Histopathological analysis of ovarian, fallopian and uterine tissues revealed significant hyperaemia, mild to moderate haemorrhage, oedema and neutrophil leucocyte infiltrations in ovarian tissue, endometrial damage and inflammatory cell infiltrations in uterine tissue and cilia loss in fallopian tubes.

Granulocyte colony-stimulating factor (G-CSF) plays a crucial role in regulating the proliferation of haematopoietic cells, particularly neutrophils, in the bone marrow. It not only increases the production of neutrophils but also shortens their maturation time. This enhances neutrophil functions by promoting phagocytosis and antibody-dependent cell-mediated cytotoxicity.[47,48] Interleukin-1 (IL-1) is a proinflammatory cytokine involved in activating acute-phase plasma protein genes in hepatic cells during infection and injury. IL1-β has been reported to regulate the G-CSF-induced acute-phase response in parenchymal cells.[49] In this study, immunohistochemically increased G-CSF and IL1-β expressions, which are known to increase in acute events, increased in the damage group, supporting the histopathologic findings.

Although the implantation of the zygote into the uterus is shaped by an inflammatory process, excessive inflammation has negative effects on implantation and the progression of pregnancy.[50,51] In this intense inflammatory background, it is not possible for the sperm to fertilize the ovum and for the zygote to implant in the tissue. Moreover, it is inevitable that this condition, which also occurs in the ovarian tissue, may have a negative effect on ovum formation and ovarian reserve. It can be said that this inflammatory and oedematous tissue formed in the ovarium may reduce the reserve capacity. In addition, the fact that these results are accompanied by neutrophilic infiltration and increases in IL1-β, an acute-phase reactant, indicates that the event progresses very rapidly and the damage may start quite early.[51] The aggressive course of this picture in the tissues as a result

of the 6-h model applied in the experimental stages, and the fact that these effects can be prevented by DPG proves that the drug may also have an acute effect. This also showed that DPG may have a protective effect on tissues in addition to the indications for oral use in some other acute events. On the other hand, Cas-3 increases detected in the injury groups indicate that cellular death may be triggered in acute events, and the decrease in these expressions in DPG groups indicates that DPG may protect all three genital tissues with its antiapoptotic effect.

Cysteine proteases known as caspases cleave their substrates on the C-terminal side of aspartate, leading to membrane blebbing, DNA breakage, exposure of phosphatidylserine on the cell surface and the formation of apoptotic vesicles. Caspases play crucial biological roles not only in cell death processes like pyroptosis and apoptosis through distinct pathways but also in noncell death processes such as inflammation, dendritic pruning, cell differentiation and migration. Cas-3 is essential in apoptosis and is commonly used to evaluate this process.[52] G-CSF stimulates neutrophil production and function, making it pivotal in inflammation. While G-CSF levels generally rise throughout the body during infection and inflammation, it is also suggested that G-CSF primarily acts as a local effector.[53] IL-1β is a potent proinflammatory cytokine crucial in regulating inflammation and the pathogenesis of diseases, especially sepsis.[54] Cas-3, G-CSF and IL-1β are pivotal markers in inflammation and apoptosis. Therefore, these markers were chosen to assess organ responses in the study. This study suggests that DPG may have a protective effect on tissues in addition to the indications for oral use in some other acute events. On the other hand, Cas-3 increases detected in the injury groups indicate that cellular death may be triggered in acute events, and the decrease in these expressions in DPG groups indicates that DPG may protect all three genital tissues with its antiapoptotic effect.

In sepsis, assessing changes in cell barrier functions, intercellular connections and permeability is crucial, alongside investigating intracellular signalling, proinflammatory cytokine production and evaluating oedema and other fluid balance disorders. These assessments are vital for understanding disease progression and treatment effectiveness. Therefore, Claudin-1, NLRP3 and AQP4 markers were selected for genetic examinations to assess responses associated with these processes.[55] It is known that Claudin-1, which is examined in genetic analyses, is an intercellular connection molecule and its expression decreases when tissue integrity is disrupted. Decreased Claudin-1 levels and increased cellular permeability with impaired tissue integrity explain the oedema characterized by inflammatory cell infiltration detected in histopathological findings.[16] Moreover, the parallelism of AQP4 levels, which allow fluid passage, with the increasing inflammatory picture explains that the metabolic wastes formed at the cellular level increase for excretion.[56] On the other hand, NLRP3 pathway, an important intracellular mechanism, plays an active role especially in inflammation secondary to infection.[57] In our study, increased NLRP3 expression in ovarian tissue with LPS supported histopathologic and immunohistochemical analyses, indicating that it contributes to the expected inflammatory event. DPG treatment suppressed the expression of all three genes and protected the tissue, paving the way for future studies on these genes.

5 | CONCLUSION

In conclusion, DPG demonstrates significant protective effects against acute systemic inflammation-induced damage in the ovaries, fallopian tubes and uterus. Its antioxidant, anti-inflammatory and antiapoptotic properties are key in mitigating these effects. Specifically, DPG achieves this by decreasing Claudin-1 expression and increasing the expression of NLRP3 and AQP4 genes, thereby promoting cellular resilience and maintaining tissue integrity in the reproductive system. These findings highlight the potential prophylactic benefits of DPG in managing inflammation-related reproductive health issues.

AUTHOR CONTRIBUTIONS

Senay Topsakal, Ozlem Ozmen, Halil Asci, Abdurrahman Gulal, Kadriye Nilay Ozcan and **Bunyamin Aydin**: Editing of manuscript drafts; project planning; and oversight. **Senay Topsakal, Ozlem Ozmen** and **Halil Asci:** Conducting experiments; investigation; data collection; and analysis. All authors were involved in drafting and revising the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or nonfinancial interests to disclose.

DATA AVAILABILITY STATEMENTS

The authors confirm that the data and materials supporting the findings of this study are available in the article.

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