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Research Article
Ellagic Acid Protects Spleen Injury Against *E. coli* Infection: Role of Hypoxia-Induced Factor-1 and Antioxidant Activities *in vivo*

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Abstract

Background and Objective: The inflammation and oxidative stress *in vivo* caused by *Escherichia coli* (*E. coli*) infection are of great significance and play a crucial role in the clinical diagnosis for both injury to and damage to different organs. The present study was conducted to investigate the anti-inflammatory and antioxidant properties of ellagic acid (EA) against fatal bacterial infections *in vivo*. **Materials and Methods:** Forty mice were assigned to four groups (N = 10): Group I (control), Group II (EA only, 70 mg/kg/day for 12 days), Group III (*E. coli* infection at 1×10 CFU/kg for 7 days) and Group IV (*E. coli*+EA, treated simultaneously for 12 days). On day 13, blood and spleen samples were collected to assess Pro-Inflammatory Cytokines (IL-1β, TNF-α), Inducible Nitric Oxide Synthase (iNOS), antioxidant enzymes (SOD, GST, GSH-Px), Hypoxia-Induced Factor-1 Alpha (HIF-1α) and spleen histopathology. Data were analyzed using One-way ANOVA and Tukey's *post hoc* test ($p<0.05$). **Results:** Levels of serum Pro-inflammatory cytokines (interleukin-beta and tumor necrosis factor-alpha), inducible nitric oxide synthase, spleen antioxidant parameters and hypoxia-induced factor-1 alpha (HIF-1α) decreased in Group III after infection. The immunoreactivity of HIF-1α was noted and inspected; histopathological spleen staining showed disturbed spleen architectures with increased levels of white pulp, areas of hemorrhage, areas of necrosis and fibrosis of the trabeculae. However, biochemical markers, immunohistochemical staining and histopathological examination revealed that there were improvements in Group IV, showing the partial restoration of normal spleen architecture with no hemorrhaging and minimal necrosis and fibrosis. **Conclusion:** The results confirmed the ability of EA to protect against spleen damage and injury induced by *E. coli* via regulation of HIF-1α and reduced oxidative damage. The data analysis highlights the potential of EA as a protective agent against spleen injury and damage.

Key words: *E. coli*, ellagic acid, HIF-1, cytokines, inducible nitric oxide synthase

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Escherichia coli, also known as *E. coli*, is a pathogen that inhabits the intestines of mammals that have warm blood; it has spread widely among humans, animals and the environment[1,2]. It is one of the most significant pathogens concerning hospital infections, animal infections, food poisoning and water pollution[3,4]. Clinically, *E. coli* can cause a wide range of illnesses in people of all ages[5,6]. These illnesses include pneumonia, bacteremia, meningitis, diarrhea and urinary tract infections. The immune system is triggered to fight pathogens, which results in the recruitment of immune cells and the release of pro-inflammatory cytokines[7]. Tumor Necrosis Factor-Alpha (TNF-α) and Interleukin-Beta (IL-β) are crucial for coordinating the innate immune response[8]. TNF-α is a type of pro-inflammatory cytokine that is primarily produced by activated macrophages. It can also be used to enhance the permeability of epithelial cells and upregulate the expression of IL-6 and IL-1β, which together help eliminate invaders[9]. However, when an immune or inflammatory stimulus is present, Inducible Nitric Oxide Synthase (iNOS) can generate the majority of nitric oxide *in vivo* to protect against invasive infectious pathogens. In addition, iNOS helps activate antigen-specific T cells and enable natural killer cells to secrete TNF-α, IL-1β and IL-6 to protect against pathogen infection[10]. For future in-depth research, it is therefore convenient to understand the dynamic changes that occur during the infection process by creating a model of *E. coli* infection in mice.

An imbalance between the levels of antioxidants in the biological system and the generation of oxidants, such as free radicals, peroxide and nitric oxide, results in oxidative stress. In this case, an excess of reactive oxygen species (ROS) may cause lipids, proteins and DNA to be damaged[11]. Mutagenesis and cellular death may result from this damage if it is not repaired. Numerous biomarkers have been employed to track oxidative stress progression in these illnesses. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), for instance, are involved in the detoxification of ROS[12]. SOD which lowers O_2 concentrations, is less active in oxidative stress-related illness such as schizophrenia and chronic bacterial prostatitis[13].

A naturally occurring secondary metabolite of bioactive polyphenolic compounds, ellagic acid (EA) is present in many different plant taxa. A notable amount of EA can be found in the wood and bark of some tree species, as well as in pomegranates (*Punica granatum* L.). Ellagic acid is gaining interest due to its anti-inflammatory, anti-mutagenic, antioxidant and antiproliferative properties. Numerous *in vitro* and *in vivo* model systems have demonstrated the pharmacological effects of EA[14].

The present study investigates biochemical parameters, inflammatory cytokines and antioxidant activities to assess the protective properties of EA against severe infection induced by *E. coli*. Moreover, HIF-1α is applied to estimate immunoreactivity expression and its role in the repair of spleen cell injury *in vivo*. Additionally, the degree of injury is measured by the histopathology of the spleens for each group, which is taken into consideration to determine and explicate EA's mode of action and its protective properties in detail.

MATERIALS AND METHODS

Study area: The study was conducted and carried out on male mice, particularly Swiss albino mice, for 6 months between October, 2024 to March, 2025.

Animals: Healthy adult Swiss albino male mice (22-25 g) were obtained from King Abdulaziz University, Jeddah, Saudi Arabia. The mice were acclimatized for 5 days at the Faculty of Science, Al-Baha University, under an adapted temperature, kept in a 12 hrs light-dark cycle and a standard mouse diet ad libitum. The study followed the animal handling guidelines of the University of Al-Baha's Ethics Committee for Scientific Research.

***Escherichia coli*:** A group of 10 mice was used to determine the number of viable *E. coli* bacteria. We performed gastric gavage with 0.5 mL of diluent from a culture frozen overnight using a Perfektum stainless-steel feeding tube (Popper & Sons, Inc., New Hyde Park, NY, USA). The goal was to inoculate 1×10^6 colony-forming units (CFUs). The weight and clinical condition of the mice were monitored every day. Each mouse was given *E. coli* (1×10^6CFU/mouse) for 4 days. The number of viable *E. coli* bacteria was determined in the mice's feces using the drop plate method[15,16]. To detect *E. coli*, 10 µL of each serial dilution of the dissolved fecal samples was seeded three times onto enterococcal agar. The phosphate-buffered saline (PBS) was sterile. For 2 days, petri dishes containing enterococcal agar were incubated aerobically at +37°C in a thermostat. This was done to detect the presence of *E. coli*. Each drop's colonies were tallied, averaged and multiplied by the dilution factor while accounting for the fecal sample's weight. Counts were made of colonies that grew on differential media and displayed typical morphology.

Ellagic acid: Ellagic acid was purchased from Sigma-Aldrich Co. (476-66-4). The effective dose was 70 mg/kg/day dissolved in 0.1% of dimethyl sulfoxide. This was administered once daily via intragastric tubes for 12 consecutive days[17].

Experimental design: The mice were randomly distributed into four groups of 10 mice per group. Group I was kept as the control. Group II was treated with EA only via intragastric tubes at 70 mg/kg/day for 12 days. Group III was injected orally with *E. coli* at a dose of 1×10^6 CFU in 0.5 mL of saline every day for 7 days and did not receive any treatment. Group IV was injected orally with *E. coli* at a dose of 1×10^6 CFU in 0.5 mL of saline and treated via intragastric tubes daily with 70 mg/kg/day, starting from day 1, concurrent with *E. coli* 1×10^6 CFU in 0.5 mL of saline, for 12 days, after a high level of stool colonization in mice was established. Then, 24 hrs after the last treatment, the spleens were removed, cleaned and prepared to assess the levels of SOD, glutathione-S-transferase (GST), GSH-Px and CAT. The remaining spleens were kept in 10% formalin for immunohistochemical and histopathological assessment.

Quantification of cytokines and inducible nitric oxide synthase in mouse sera: Using commercially available kits and following the manufacturer's instructions, the Enzyme-Linked Immunosorbent Assay (ELISA) was used to measure quantities of IL-1β and TNF-α in the serum of each mouse (R&D Systems Inc., Minneapolis, MN, USA). Following the manufacturer's instructions, 100 µL of serum was diluted and IL-1β- and TNF-α-specific biotinylated monoclonal antibodies were added for incubation. Streptavidin-HRP was added after washing. By utilizing 492 nm as the primary wavelength, an ELISA reader (BMG, Germany) evaluated the absorbance[18]. The iNOS levels were determined according to the instructions of the ELISA kit (NeoBioscience, Shenzhen, China)[19].

Determination of SOD, GST and GSH-Px activity in spleen homogenates: Each mouse's spleen was dissected, cleaned in PBS and homogenized on ice in a buffer containing 0.05 M Tris-HCl pH 7.9, 25% glycerol, 0.1 mM EDTA and 0.32 M $(NH_4)_2SO_4$, supplemented with a protease inhibitor tablet (Roche). Homogenization was performed using a Polytron homogenizer. This was followed by sonication in an ice bath for 15 sec to prevent overheating. Next, they were centrifuged at 12,000 rpm at 4°C for 5 min. A BCA kit (Pierce, Rockford, USA) was used to measure protein concentration after the supernatant was divided into smaller portions and kept at -80°C. A lysis buffer with diluted albumin was used as the standard. The SOD, GST and GSH-Px levels were measured using established protocols[20].

Immunohistochemical examination of hypoxia-induced factor-1 alpha: Immunohistochemistry was used to assess the expression of HIF-1α in all mouse groups. The prepared sections were covered for 1.5 hours with ideally diluted anti-HIF-1α mouse antibodies (Sigma-Aldrich) after being rinsed in PBS. The sections were then washed and treated for 30 min with a secondary antibody tagged with a poly-horseradish peroxidase enzyme (Sigma-Aldrich). The slides were treated with a freshly prepared 3,3 -Diaminobenzidine tetrahydrochloride solution for 5 min, washed and treated with Mayer's hematoxylin, dehydrated in graded alcohol (50, 70, 90 and 100%), cleared with xylene and mounted using a non-aqueous permanent mounting medium, as described previously by Vaughan *et al.*[21].

Histopathological examination of the spleen: The spleen specimens were dehydrated, embedded in paraffin, fixed in 10% paraformaldehyde and sectioned at a thickness of 5 µm. The sections were stained with Hematoxylin and Eosin (H&E). Morphological changes were examined using an Eclipse 80i microscope (Nikon) and images were captured with a DS-Fi1 digital microscope camera (Nikon)[22]. Tissue slides with either H&E or immunohistochemical staining were photographed using an MVV5000CL digital eyepiece installed on a MEIJI MX5200L microscope and Future WinJoe software, with 10× and 40× objectives. The resulting 10× immunohistochemical images were analyzed on an Intel® Core i7-based computer using Fiji ImageJ (version 1.51r; NIH, Maryland, USA) software. To measure the staining percentage of the surface area, the color deconvolution 2 plugin (for digitally separating histological dyes) was used. This provided three independent digital images (H&E, DAB and a complementary image). Five random fields measuring 200×200 µm from each slide were analyzed.

Statistical analysis and data interpretation: Data was fed to the computer and analyzed using GraphPad Prism 8 (GraphPad Software). Percentages of immunohistochemical positive areas were normally distributed according to tests of normality (Shapiro–Wilk tests). Normally distributed data were presented as Mean and Standard Deviation (SD) values.

One-way ANOVA tests, followed by *post-hoc* Tukey's multiple comparison tests, were used to compare the normally distributed data groups. The significance of the obtained results was judged at the $p<0.05$ level.

RESULTS

Assessment of inflammatory mediators and inducible nitric oxide synthase in mouse sera: In the current study, the pro-apoptotic cytokines and inflammatory mediators, including IL-1β and TNF-α, exhibited an elevation in Group III compared to Group I, at 68.89±1.09 ng/mL and 2229.91±15.67 ng/mL, respectively. Group IV, treated with EA, displayed significant improvements in both IL-1β and TNF-α. These levels almost matched those of the control group, Group I. The levels of IL-1β and TNF-α in the treated group, Group IV, decreased significantly to 32.52±1.39 and 407.73±7.44 ng/mL, respectively. Moreover, iNOS is an enzyme involved in inflammatory responses; it may cause the production of sustained high levels of nitric oxide. Group III, infected with *E. coli*, showed 3-4-fold increases compared to Group I. Group IV, treated with EA, displayed significant improvement in terms of iNOS, with levels almost matching the control group. The level of iNOS in the treated group (IV) decreased significantly (Table 1).

Antioxidant activities in the spleen homogenates of different mouse groups: Group III showed a significant decrease in antioxidant enzyme activities for SOD, GST and GSH-Px compared to Group I. When *E. coli* was injected, it was dose-dependent, with notable alterations at 1×10^6 CFU. Specifically, SOD levels were 21.60±1.09 U/mg in Group III compared to 48.72±1.84 U/mg in Group I; GST activity was 34.44±0.80 U/mg in Group III, which was significantly lower than in Group I (74.77±0.19); GSH-Px activity was 40.29±0.95 U/mg in Group III, significantly lower than 74.77±0.19U/mg in Group I (Table 2). Treatment with EA (at a dose of 70 mg/kg bw) in Group IV significantly increased the activity levels of SOD, GST and GSH-Px compared to Group III ($p<0.0001$) (Table 2). No significant changes in antioxidant activity were observed in Group II in comparison to Group I. Additionally, there were no signs of agitation, instances of weight loss, sores or mortality in Group II, which received EA treatment.

Immunohistochemical detection of hypoxia-induced factor-1 alpha results (light microscopic examination): Figure 1(a-h) displays the immunohistochemical staining of HIF-1α in spleen tissues at 10× and 40× magnifications. In the control (a, e) and EA-only (b, f) groups, spleens exhibited normal architecture with a large positively stained red pulp and negligible or absent staining in the white pulp, indicating low hypoxic stress. In the *E. coli*-infected group (c, g), strong positive staining of HIF-1α was detected, especially in the expanded white pulp area, accompanied by reduced red pulp, reflecting inflammation-induced hypoxia. In the *E. coli*+EA-treated group (d, h), partial recovery was evident, with moderately positive red pulp areas and improved tissue organization, demonstrating EA's regulatory effect on HIF-1α expression and hypoxic response.

The percentages of HIF-1α staining surface areas for all groups are presented in Table 3.

Table 1: Serum inflammatory mediators and inducible nitric oxide synthase in different groups

Group (Mean±SD)	Control group	Control-treated EA group	Infected *E. coli* group	Infected *E. coli* treated EA group	p- value	f- value
Serum IL-1β level (ng/mL)	26.17±0.97	26.00±1.57	68.89±1.09^{ab}	32.52±1.39^{abc}	p<0.0001	2585
Serum TNF-α level (ng/mL)	144.80±0.85	146.18±1.31	2229.91±15.67^{ab}	407.73±7.44^{abc}	p<0.0001	133517
Serum iNOS level (ng/mL)	9.11±0.68	10.68±0.63	34.50±0.91^{ab}	13.13±0.60^{abc}	p<0.0001	1555

Values expressed as Mean±SD, Used test: One-way ANOVA followed by *post hoc* Tukey's multiple comparison test, a: Significance vs. control, b: Significance vs. control treated EA and c: significant vs. infected *E. coli* at p<0.05

Table 2: Antioxidant activities SOD, GST and GSH-Px in spleen homogenate of different groups

Group (Mean±SD)	Control group	Control-treated EA group	Infected *E. coli* group	Infected *E. coli* treated EA group	p-value	f-value
SOD activity (U/mg protein)	48.72±1.84	49.06±0.73	21.60±1.09^{ab}	51.84±1.45^{abc}	p<0.0001	1122
GST activity (U/mg protein)	74.77±0.19	75.05±0.54	34.44±0.80^{ab}	63.25±2.08^{abc}	p<0.0001	2748
GSH-Px activity (U/mg protein)	81.08±4.76	83.44±6.54	40.29±0.95^{ab}	65.48±1.77^{abc}	p<0.0001	226.8

Values expressed as Mean±SD, Used test: One-way ANOVA followed by *post hoc* Tukey's multiple comparison test, a: Significance vs. control negative, b: Significance vs. control negative treated EA and c: Significant vs. infected *E. coli* at p<0.05

Table 3: Staining surface area percentage of the Immunohistochemistry HIF-1α in different groups

Group	Control group	Control-treated EA group	Infected *E. coli* group	Infected *E. coli* treated EA group	p-value	f-value
HIF-1α IHC staining % mean	67.77	70.38	30.54^{ab}	45.55^{abc}	p<0.0001	55.23
SD	±7.00	±5.88	±6.59	±12.58		

Values expressed as Mean±SD, Used test: One-way ANOVA followed by *post hoc* Tukey's multiple comparison test, a: Significance vs. control negative group, b: Significance vs. control negative treated EA group and c: Significant vs. infected *E. coli* group at p<0.05

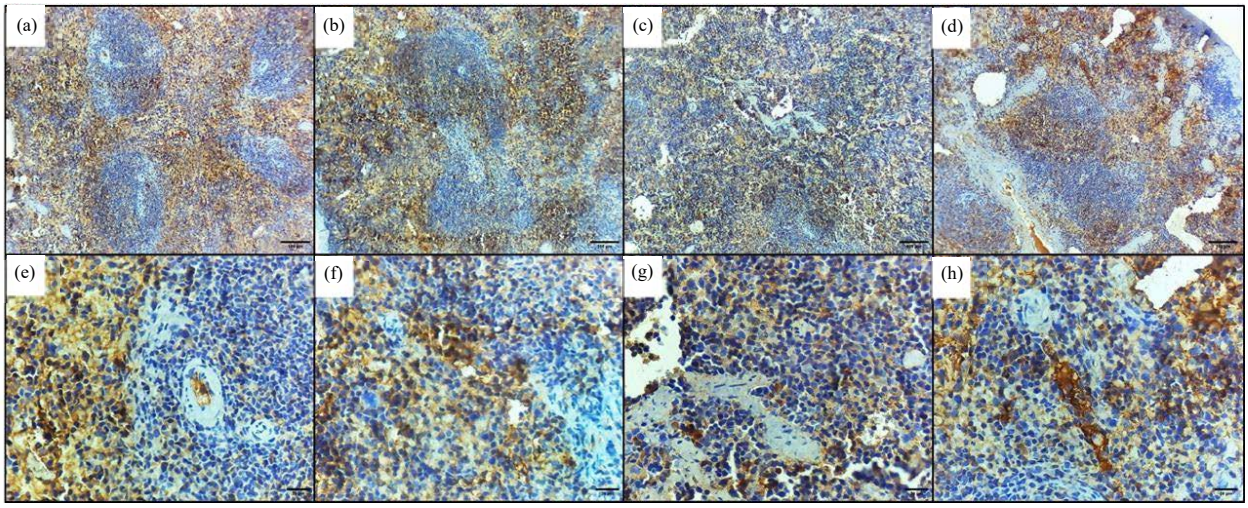

Fig.1(a-h): HIF-1α immunohistochemical staining (10× and 40×), (a) Control spleen showing negative HIF-1α expression with normal red and white pulp (10×), (b) EA-only group with similar architecture and negative HIF-1α staining (10×), (c) *E. coli*-infected group showing positive HIF-1α expression, increased white pulp, and reduced red pulp (10×), (d) *E. coli* + EA-treated group showing moderate HIF-1α expression and partial red pulp restoration (10×), (e) Control spleen confirming negative HIF-1α and intact architecture (40×), (f) EA-only group with normal features and minimal nuclear staining (40×), (g) *E. coli*-infected spleen showing intense HIF-1α staining and disrupted tissue (40×) and (h) High magnification (40×) of *E. coli*+EA-treated spleen showing improved structure and reduced HIF-1α signal (40×)

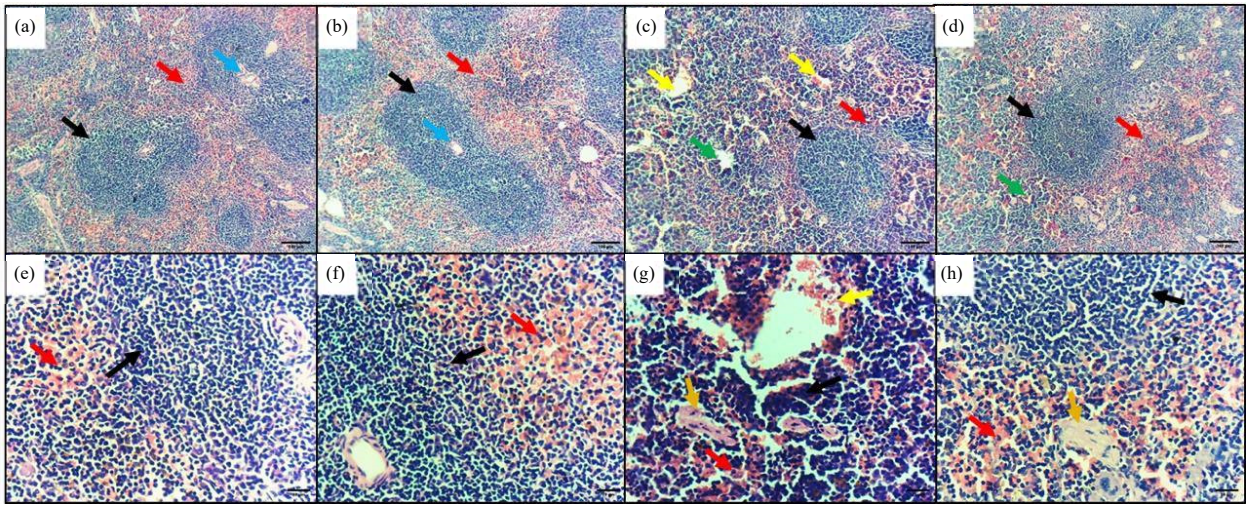

Fig. 2(a-h): H&E-Stained spleen sections (10× and 40×), (a) Control group showing normal white-to-red pulp ratio, no pathology (10×), (b) EA-only group with preserved spleen architecture, no lesions (10×), (c) *E. coli*-infected group showing white pulp expansion, hemorrhage, necrosis, and fibrosis (10×), (d) *E. coli* + EA-treated group showing partial restoration with reduced lesions (10×), (e) Control spleen with intact red and white pulp areas (40×), (f) EA-only spleen with no signs of hemorrhage or necrosis (40×), (g) *E. coli*-infected spleen showing hemorrhage (yellow arrow), necrosis (green arrow) and fibrosis (orange arrow) (40×) and (h) *E. coli* + EA-treated spleen showing minimal fibrosis and balanced pulp regions (40×)

Histopathological results (light microscopic examination of H&E-stained sections): Figure 2(a-h) presents the H&E-stained spleen sections at 10× and 40× magnifications. The control (a, e) and EA-only (b, f) groups showed intact spleen histology, normal white and red pulp ratios and absence of pathological alterations. Severe disruptions were observed in the *E. coli*-infected group (c, g), characterized by increased white pulp, hemorrhage (yellow arrows), necrosis (green arrows) and fibrosis of trabeculae (orange arrows). The red and black arrows represent red and white pulp, respectively. In the EA-treated infected group (d, h), the spleen architecture was partially restored, showing balanced red and white pulp, no hemorrhage and minimal necrosis or fibrosis, indicating the histological protection conferred by EA.

DISCUSSION

Gram-negative *E. coli* is one of the most significant pathogens in causing hospital infections, animal infections, food poisoning and water pollution[23,24]. It is considered a member of the normal intestinal microbiota. However, some *E. coli* spreads widely among humans, animals and the environment[25,26]. Clinically, *E. coli* can cause a wide range of illnesses, such as pneumonia, septicemia, meningitis, diarrhea, urinary tract infections and bacteremia[27,28]. Public health is greatly impacted by pathogenic *E. coli* in terms of morbidity and mortality.

Drug-resistant *E. coli* has become a significant and severe public health concern in recent years[29]. As a result, clinics must deal with the restrictions and passive states of *E. coli* medications. Here, to better understand the *in vivo* infection caused by *E. coli* in clinics and establish a theoretical basis for drug research and development, the disease process and related factors were studied using mice infected with *E. coli*.

When *E. coli* was injected into the mice for the study, we noted that the infection dose was 1×10^6 CFU/kg. On the first, fourth and seventh day of *E. coli* injection, mouse tissues were obtained for colony counting. All organs had varying degrees of infection; those collected on the seventh day had the highest concentrations of contaminated bacteria. The subsequent experiment was made possible by the administered dose of *E. coli* and the bacterial invasion of the mice's tissues following the injection of *E. coli*.

Innate immune recognition is widely known to be the cause of both non-inflammatory phagocytosis of apoptotic cells and inflammatory phagocytosis of harmful bacteria[30]. The level of the body's immunological response in mice can be reflected in the concentration of inflammatory substances. TNF-α and IL-1β are two of the most potent pro-inflammatory factors

In this study, the administration of *E. coli* to mice (Group III) resulted in a significant increase in the serum levels of IL-1β, TNF-α and iNOS compared to the control group (I). These results align with those of Barrientors *et al*.[31], which revealed that *E. coli* can significantly elevate the pro-inflammatory cytokine levels in the liver and spleen 4 days after *E. coli* infection. Similarly, Long *et al*.[32] found that infecting mice with *E. coli* caused an increase in the concentrations of IL-6, IL-β and TNF-α, which peaked on the seventh day. However, the administration of the EA in Group IV for 12 days significantly reduced the serum levels of IL-1β, TNF-α and iNOS compared to those of the infected group (Group III) ($p<0.001$) (Table 1). This finding is consistent with the research of Urfalıoğlu *et al*.[33], who demonstrated that EA reduced pro-inflammatory cytokine activity, such as IL-1β and TNF-α activity. This is explained by the anti-inflammatory properties of EA. Similarly, Kim *et al*.[34] demonstrated that by inhibiting the activation of NF-κB/MAPK pathways in gut, liver and brain injuries, EA co-treatment significantly reduced the amount of pro-inflammatory and oxidative stress markers. These findings imply that EA merits more research as a possible remedy for inflammatory illnesses because it is effective in reducing Inflammatory Bowel Disease (IBD) in mice. According to Gupta *et al*.[35], further research on EA may clarify its function in inhibiting the expression of iNOS.

In the present study, significant decreases ($p<0.05$) in SOD, GST and GSH-Px levels were observed in the spleen homogenates of mice in Group III following *E. coli* infection at a dose of 1×10^6 CFU compared to Group I (Table 2). These results are consistent with Long et al[32] results, as they also reported that *E. coli* can induce oxidative stress and inflammation in mice, resulting in varying degrees of tissue damage to the kidney, lungs and spleen. This finding lends theoretical support to the pathological and inflammatory changes brought on by *E. coli* infection *in vivo*. Moreover, our results agree with Halwani's findings[36], which demonstrated that the administration of *E. coli* led to decreased SOD and GSH-Px levels in the intestines of mice, indicating oxidative damage to mice organs caused by *E. coli* at a dose of 1×10^7 CFU. In contrast, Group IV, treated with EA, exhibited increased antioxidant activity. This is in agreement with Xu *et al*.[37], who reported that EA supplementation promoted the lipid metabolism and antioxidant capacity to maintain the liver health of mice. Xu *et al*.[38], also found that the administration of EA can enhance the growth of mice, promote intestinal

development, increase antioxidant capacity and regulate the intestinal microbiota. These data revealed the antioxidant activity of EA, as in parallel with Kumar *et al*.[39], who reported that EA at a dose of 50 mg/kg improved levels of circulating testosterone (increased 3βHSD) and decreased oxidative stress.

The transcription factor, HIF, is crucial for cellular responses to low oxygen levels because it triggers a metabolic switch that enables cells to endure such a situation. Since inflammatory and infected tissues are frequently hypoxic during immunity, HIF aids immune cell adaptation. Stabilization of HIF-1α can also occur in normoxic conditions during inflammation and immunity. Our results demonstrated that the *E. coli*-infected group (III) had positive HIF-1α expressions in spleens, showing mildly disturbed spleen architectures, increases in white pulp areas and reductions in positive red pulp areas, indicating the activation of inflammatory pathways in response to *E. coli* infections. The results are similar to those obtained by Groneberg *et al*.[40], who stated that *E. coli* infections increase the amount of HIF-1α in the liver, which then induces the differentiation of Th17 cells by increasing IL-6 expression. Our results are also in agreement with Santos *et al*.[41], who stated that HIF-1α is stimulated by pro-inflammatory cytokines, growth factors and a wide range of infections. Its induction is a general element of the host's response to infection. In contrast, Group IV, which was infected by *E. coli* and treated with EA, exhibited modulation responses to the expression of HIF-1α, showing partial restoration of normal spleen architectures characterized by moderate positive surface area of red pulp (Figure 1D). The values of HIF-1α staining surface area percentage. These results are in consistent with Nakai *et al*.[42], which revealed that EA has HIF-1α-suppressive effects on the human bladder carcinoma cell line (ECV304). Furthermore, our findings are consistent with those of Duan *et al*.[43], who confirmed that EA has a role in the reduction of HIF-1α and AMP-activated protein kinase (AMPK) in lung cancer cells. Moreover, administering EA to mice harboring tumors resulted in a significant reduction in tumor growth, elevation in p-AMPK levels and suppression of HIF-1α levels. These results point to EA as a potentially effective chemotherapeutic agent that targets the mitochondrial metabolism of lung cancer .

Furthermore, the histopathological outcomes indicated that the spleen injuries exhibited distinct histopathological changes in the experimental group (III) in the form of disturbed spleen architectures, with increases in white pulp, areas of hemorrhage, areas of necrosis and fibrosis of the trabeculae. These fallouts are consistent with the results of Long *et al*.[32], who illustrated that spleen, lung and kidney tissues can sustain varying degrees of damage due to *E. coli*-induced inflammation and oxidative stress in mice. This provides theoretical support for the pathological and inflammatory changes that result from *E. coli* infection *in vivo*.

Concomitant administration of EA and *E. coli* in Group IV resulted in limited signs of spleen injury, as the specimens showed partial restoration of normal spleen architectures, equal white to red pulp ratios, no hemorrhages and minimal necrosis and fibrosis. These findings are in accordance with Lei *et al*.[44]. Their study's histopathological observations demonstrated that EA was useful in treating spleen abnormalities, including blood stasis in red pulp, lymphocyte necrosis, localized central dilatation of spleen nodules and thickening of the marginal area. These findings demonstrated the advantageous effects of EA in terms of the protection of spleen tissue against toxicity changes.

Taken together, these results suggest that EA has potent anti-inflammatory and antioxidant properties. The experimental data supports the idea that EA could potentially be useful in the control of spleen damage and/or injury in experimental models. This is accompanied by its improvements to antioxidant parameter activities and regulation of HIF-1α expressions.

CONCLUSION

The present research strongly suggests that EA is a powerful agent in protecting the spleen from damage induced by *E. coli* infection. Furthermore, this study's data revealed that spleen damage is severely influenced by *E. coli* infection. The EA treatment resulted in improvements to pro-inflammatory cytokine levels, antioxidant activity that controls HIF-1α levels and pathological spleen outcomes. These results imply that EA offers exceptional protection against spleen injury and/or damage, which indicates that EA is a strong candidate as a preventive agent for spleen harm. Subsequent research should better define the mechanisms behind the protective properties of EA. In addition, further investigations and research should focus more on the biological and pharmacological properties of EA, which may indicate whether it can have future applications in human clinical trials.

SIGNIFICANCE STATEMENT

The present study was conducted to investigate the anti-inflammatory and antioxidant properties of ellagic acid (EA) against fatal bacterial infections, Escherichia coli, *E. coli*,

in vivo which has spread widely among humans and animals. In addition, the present study clearly showed the improvement of antioxidant parameters, pro-inflammatory cytokines after administration of ellagic acid in induced infected mice. These results implicated that EA offered exceptional protection against spleen injury and/or damage, which indicates that EA is a strong candidate as a preventive agent for spleen harm. Additionally, the histopathology and immunohistochemistry assured the data gained in the present work. Such results may be impressive in the future in using this EA the considering further research work.

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