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Natural immunomodulation of host defense against *Klebsiella aerogenes*: evidence from Licorice extract

Roua J. Mohammed

* Corresponding author. Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq. E-mail: ruaa.jassem1103a@covm.uobaghdad.edu.iq

Ikram Abbas Al Sammaraa

Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq. E-mail: ikram@covm.uobaghdad.edu.iq

Sabrin Ibraheem Mohsin

Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq. E-mail: Sabrin.i@covm.uobaghdad.edu.iq

Abstract

Objective

The emergence of *Klebsiella aerogenes* as a possible pathogen has been linked with both gastrointestinal and systemic infections in animals and human beings. Antimicrobial resistance among strains of *Klebsiella* species has increased. Thus, there is a need for seeking alternative sources of immunomodulating substances. Hence, the objective of this study was to evaluate the immunomodulatory and prophylactic effects of Glycyrrhiza glabra (licorice extract) on mouse models of *Klebsiella aerogenes* infection.

Materials and methods

Samples were obtained from diarrhea cases of 50 cats for culturing and identification of *K. aerogenes*. These bacteria were then used in experimentally infecting 48 mice of the Swiss albino strain. A total of six groups of eight mice each were included. Three groups were given oral licorice extract at various doses of 50, 150, and 250 mg/mL. Two control groups comprising of negative controls (oral administration of PBS) and positive control. An additional group was only provided with extract but not infected. Licorice extract was administered twice weekly for 21 days followed by injection with *Klebsiella aerogenes* bacteria at 1.5×10^8 CFU/mL.

Results

Infected untreated mice had significant increases in inflammatory and hematological indices compared to negative control mice ($P \leq 0.05$). A significant rise in white blood cells' count

occurred in infected mice, whose mean value amounted to 12.8 ± 1.4 ×10³/µL. While those in control group were lower at 6.2 ± 0.9 ×10³/µL. Moreover, IL-6 levels in infected mice significantly increased to 85.6 ± 5.3 pg/mL versus 28.4 ± 3.1 pg/mL in controls. Infected untreated mice experienced severe inflammatory and degenerative histopathological lesions. On the other hand, infected but licorice-treated mice exhibited reduced inflammation, IL-6 levels, improvements in hematological indices, and lesser degree of tissue damage. The lowest dose (50 mg/mL) was shown to produce maximum protective effect, implying that there is no clear relationship between the concentration of licorice and its biological impact on experimental mice.

Conclusion

Glycyrrhiza glabra extract produced certain anti-inflammatory and protective effects against infection caused by *Klebsiella aerogenes* in mice. Licorice extracts decreased inflammation and improved pathological alterations observed in infected animals. Glycyrrhiza glabra may be regarded as an interesting candidate for development as a natural immunomodulator; nonetheless, more research needs to be done.

Keywords: immunomodulation, IL-6, *Klebsiella aerogenes*, licorice extract

Paper Type: Research Paper.

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Introduction

The *Klebsiella aerogenes* is an opportunistic Gram-negative bacterium which is currently being increasingly identified as the cause of diseases affecting humans as well as animals, mainly involving the gastrointestinal and systemic diseases (Custodio et al., 2020; Wesevich et al., 2020). The bacterial species can adhere to mucosa surfaces and produce virulent determinants such as capsular polysaccharides, endotoxins, and adhesion factors (Hu et al., 2025). As for clinical cases in veterinary medicine, *Klebsiella aerogenes* bacteria have been detected in animals infected with diarrhea and various other infections (Ribeiro et al., 2022). Infections induced by *K. aerogenes* trigger a series of immune reactions that incorporate innate and adaptive immunity mechanisms

(Gowripriya et al., 2024). Some of the major mediators of the immune reaction to this infection include interleukin-6 (IL-6). This is a pro-inflammatory cytokine that is instrumental in inflammation regulation, immune cell activation, and acute phase reaction induction (Gowripriya et al., 2024). Increased production of IL-6 is correlated with the seriousness of bacterial infections due to the level of immune system activation during such infections (Shanshal et al., 2023). Similarly, the CBC analysis can be utilized as an indicator for various systemic reactions to bacterial infections, including leukocytosis and neutrophilia (Alexandru et al*.,* 2025). Nonetheless, overreactions of inflammation can cause tissue injury and worsen disease severity (Alfaro et al., 2022). Thus, manipulation of immune responses becomes a key point of interest in treatment. In this regard, medicinal herbs like licorice (Glycyrrhiza glabra) have become popular because of their immunomodulatory, anti-inflammatory, and antimicrobial activities (Leite et al., 2022). The phytochemicals found in licorice extract include glycyrrhizin and flavonoids, which play a role in regulating cytokine secretion, particularly interleukin-6 (IL-6), and correcting hematological profiles by optimizing immune responses and lowering oxidative stress (Zhong et al., 2025; Pastorino et al., 2018). *Klebsiella* species have attracted growing attention in recent years due to their significance as newly emerged infectious agents in veterinary practices, linked with drug resistance and transmission to humans (Mihu et al., 2026; Martin et al., 2025; Ibraheim et al.,202٤). Nevertheless, existing literature has centered around *K. pneumoniae* and other *Klebsiella* species, with fewer studies dedicated specifically to *K. aerogenes*. Few researchers have explored the possible link between feline diarrhea and the consequent changes in their immune response and blood parameters (Mihu et al., 2026; Martin et al., 2025; Ibraheim et al.,2024). Furthermore, there is not enough research on using natural immunomodulators like licorice extract to regulate the IL-6 level and other hematological indicators in cats infected by *Klebsiella* aerogenes. Consequently, this study aimed to investigate the possible function of licorice extract as a natural immunomodulator in feline infections with *K. aerogenes*.

Material and methods

Samples collection and isolation of bacteria: A total of 50 fecal specimens from the diarrheic cats were subjected to isolation and characterization of Klebsiella aerogenes. The isolated bacterial strain was subsequently used to experimentally infect 48 Swiss albino mice for assessing the immunomodulating activity of the licorice extract. The samples were transported to the laboratory in a cold box after a maximum of 2 hours. Each sample was put in a sterile test tube containing 1g of sample, 10 mL normal saline and 0.1mL of sample suspension. This mixture was inoculated on Mac Conkey agar (HiMedia, India) and EMB agar (HiMedia, India).

Incubation was done at a temperature of 37°C for 24 to 48 hours. Diagnosis of bacteria by Vitek 2 compact system.

Extraction of licorice root fluid extract: Licorice Root Fluid Extract was obtained as a nutritional supplement from NATURE'S ANSWER (Dosage: 2000 mg, free from alcohol). Different amounts of Licorice Root Fluid Extract were prepared including 250 mg, 150 mg, and 50 mg.

Experimental design: The experiment consisted of two phases: 1- Isolation of *Klebsiella aerogenes* bacteria from 50 diarrheic cats and 2- Infection of 48 mice of the Swiss albino strain with *Klebsiella aerogenes* to determine the effect of licorice extract on immunity. Forty-eight Swiss albino mice (17-18 g) were randomly divided into six groups (8 mice per group):

Groups G1-G3 (Treatment Groups): Treated with glycyrrhiza extract at 250, 150, and 50 mg/mL orally twice a week for 21 days.

Group G4 (Negative control): Non-infected + treated with PBS.

Group G5 (Positive control): Infected, no treatment /orally infected with *Klebsiella aerogenes* at 1.5×10^8 CFU/mL.

Group G6 (Extract control): Non-infected + treated with glycyrrhiza extract at 150 mg/mL orally twice a week for 21 days.

Treatment procedure: Glycyrrhiza extract was orally given to mice, twice a week for 21 days.

Infection procedure: After 21 days from treatment, mice in groups G1-G3 and G5 were orally infected with *Klebsiella aerogenes* at 1.5×10^8 CFU/mL concentration. Samples were taken from blood and organs (liver, spleen, lung and intestine) on days 21 and 7 day from infection. Blood samples were collected for CBC analysis, and the IL-6 levels were assessed by using ELISA kit (Elabscience, China) for mouse IL-6 according to the manufacturer's protocol. Optical density was measured at 450 nm (Socimed Sarl, France), and the concentrations were estimated from the standard curve.

Infectious dose: The first study conducted was used to determine the infectious dose of the *K. aerogenes* through the McFarland method involving the creation of three different dilutions (1.5×10^8, 3.0×10^8, and 6.0×10^8 CFU/mL) from twenty-four mice which were divided into three groups. Group 1 received 1.5×10^8 CFU (0.1 mL) orally. While, Group 2 was injected with 3.0×10^8 CFU (0.1 mL orally). Group 3 mice were also orally administered 3.0×10^8 CFU (0.1 mL). clinical signs detected from 6 hour from infection to 7 days to evaluate the standard ID (Saganuwan, 2011).

Histopathological examination: The organ tissues collected from the liver, lungs, spleen, and intestines after placing the specimens in a 10% formalin solution. The lesions were rated

using a four-grade scale that included the following: 0 = normal; 1 = mild; 2 = moderate; and 3 = severe. The grading was done depending on the extent of inflammation and tissue injury, with an average score calculated for each group. This approach was carried out following the procedures outlined in the literature (Snyder et al., 2022).

Statistical analysis: Effects of groups and time on variables have been evaluated using Statistical Packages of Social Sciences – SPSS. In this research, the means of data were tested using Least Significant Difference Method (LSD) to determine the significance between means. Differences among groups were determined using One-Way Analysis of Variance (ANOVA) test with LSD as Post-hoc Test (George and Mallery, 2019).

Results

Isolation: The study on 50 cat feces from cases of diarrhea found that 2 samples were positive for *K. aerogenes*, constituting 4%, as illustrated in (Table 1).

Table 1. Prevalence rate of *K. aerogenes* isolates.

Months of study	Samples No.	Isolates No.	Positive percentage %
September 2025 to February 2026	50	2	4%

Identification: All *K. aerogenes* strains were identified as forming purple colonies on EMB agar. On MacConkey agar, they formed mucoid pink colonies because of lactose fermentation (Figure 1).

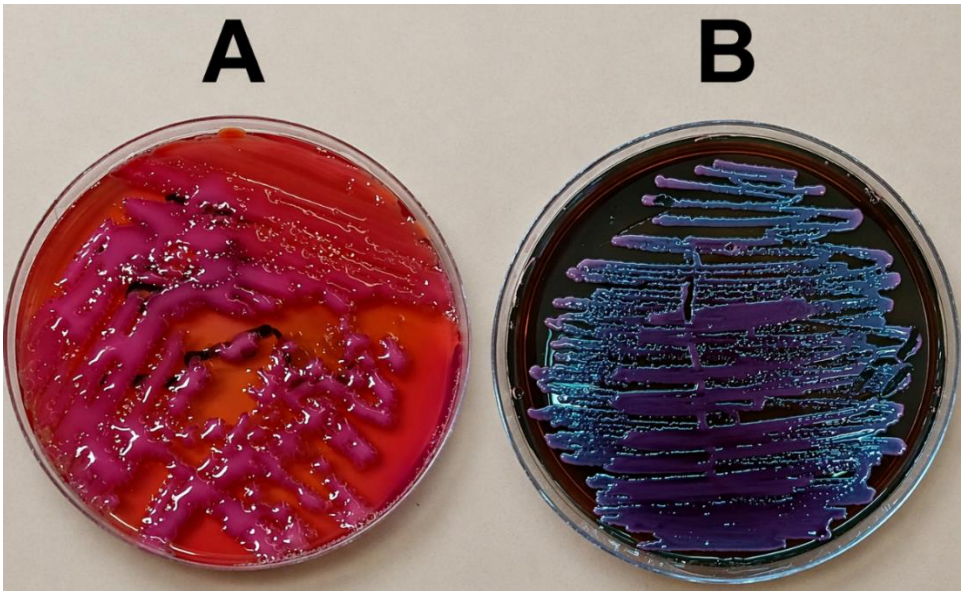

Figure 1. *K. aerogenes* on MacConkey agar (A), and EMB (B)

Vitek 2 compact system: The Vitek 2 Compact system was used to of *K. aerogenes*, yielding a 99% probability of identification, as illustrated in (Table 2).

Table 2. Vitek 2 compact system for identifying *K. aerogenes*

Identification Information	**Analysis Time: 3.90 hours**	**Status: Final**
Selected Organism	99% Klebsiella aerogenes	Bionumber: 6605734652164210

Biochemical details

2	APPA	-	3	ADO	-	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

Phytochemical analysis of Licorice root extract: The Licorice root extract was subjected to qualitative phytochemical screening to identify the presence of major bioactive constituents. The results of the analysis are presented in Table 3.

Table 3. Qualitative analysis of phytochemical of Licorice root extract

No.	**Test**	**Licorice root extract**
1	Tannins Test	+
2	Carbohydrates Test	+
3	Glycosides Test	+
4	Phenols Test	+
5	Resins Test	-
6	Flavonoids Test	+
7	Saponin Test	+
8	Alkaloid Test	-
9	Protein Test	-
10	Coumarins Test	-
11	Terpenes Test	-
12	Steroids Test	-

Determination of Interleukin-6 (IL-6): Statistically significant effects have been found after the experimental groups ($p < 0.001$). For instance, on day 7 after infection, the value for the positive control group of *Klebsiella aerogenes* was 330.45 ± 3.93 pg/mL. It is significantly high

relative to all other groups and confirms that the infection was successfully induced (Table 4). Contrarily, all groups receiving treatment registered a significant decrease in the value of the measured parameter. The highest concentration (250 mg/mL) recorded G1 (182.49 ± 0.37 pg/mL), and the next was G2 (150 mg/mL with 171.89 ± 0.50 pg/mL). The moderate decrease was noted in G3 (50 mg/mL) and G6 (150 mg/mL), which had the value of 122.27 ± 0.46 and 121.25 ± 0.44 pg/mL, respectively. These were not significantly different from each other. The negative control had no change (82.06 ± 0.28 pg/mL). On the 21st day after infection, G1 still had a value that was significantly higher (121.95 ± 0.52 pg/mL) relative to other groups. Whereas, there were no significant differences between G2, G3, G4, G5, and G6. Also, there were significant differences between pre-infection and post-infection values within all groups except the negative control (G4) (Table 4).

Table 4. IL-6 concentrations (pg/mL) in experimental groups

Group (n=8)	**After 21 Days** from treatment **(Mean ± SE)**	**After Infection (Mean ± SE)**	**p-value (Within Group)**
G1 (250 mg/mL)	121.95 ± 0.52^{a}	182.49 ± 0.37^{b}	$p < 0.001$
G2 (150 mg/mL)	80.66 ± 0.17^{a}	171.89 ± 0.50^{b}	$p < 0.001$
G3 (50 mg/mL)	81.03 ± 0.25^{a}	122.27 ± 0.46^{b}	$p = 0.002$
G4 (Control −)	81.43 ± 0.48^{a}	82.06 ± 0.28^{a}	$p = 0.721$
G5 (Control +)	82.88 ± 0.65^{a}	330.45 ± 3.93^{c}	$p < 0.001$
G6 (Extract control)	81.02 ± 0.24^{a}	121.25 ± 0.44^{b}	$p = 0.003$

The values are presented as mean ± SE. Lowercase letters (a-c) in the same row show statistically significant differences at various time intervals ($p < 0.05$). The differences among the groups were analyzed by one-way ANOVA followed by the LSD post hoc test.

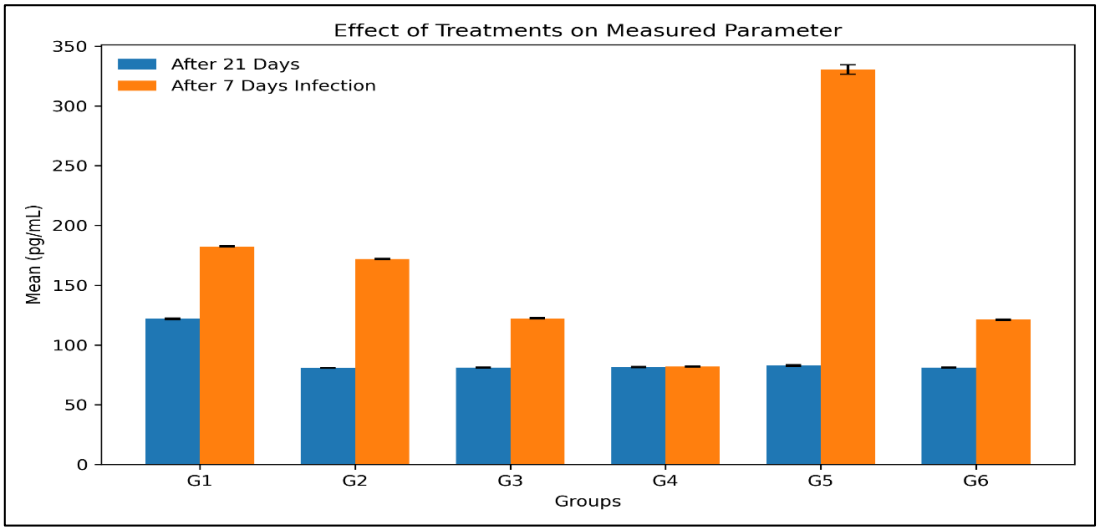

Figure 2. IL-6 concentrations (pg/mL) in experimental groups

Complete blood count (CBC): According to the hematological investigation results, there have been certain changes in the experimentally treated groups in relation to the state after infection and therapy. In the case of a WBC count, a significant growth in the positive control group (G5) occurred (14.7). It suggests an intensive activity of immunity in response to infection. The negative control group (G4) was characterized by relatively low WBC numbers (3.9). Treated groups had moderate WBC counts, such as in G6 (10.8) and G2 after infection (8.7). The number of lymphocytes (LYM#) was considerably high in G5 (13.2). Treatment resulted in a reduction of LYM#. Thus, it is possible to observe lower values in G6 (8.5) and G2 after infection (8.1). Monocyte (MON#) and granulocyte (GRA#) counts follow the same pattern as WBC counts. Because they are significantly high in infected untreated groups (G5) and lower in groups with treatment. When it comes to red blood cell count (RBC), its decrease was noticed in G1 after infection (6.81). Most of the treated groups had RBC values close to normal ones (Table 5).

Table 5. Hematological analysis

Parameter	G1 (250 mg/mL)	G1 After-Infection	G2 (150 mg/mL)	G2 After-Infection	G3 (50 mg/mL)	G3 After-Infection	G4 C-	G5 C+ (k)	G6 C+ (E)
WBC	7.7	5.2	11.2	8.7	8.1	9.4	3.9	14.7	10.8
LYM#	7.1	4.9	8.9	8.1	7.5	8.6	3.6	13.2	8.5
MON#	0.3	0.1	1	0.2	0.3	0.4	0.2	0.8	1
GRA#	0.3	0.2	1.3	0.4	0.3	0.4	0.1	0.7	1.3
RBC	9.15	6.81	8.09	8.58	7.98	8.25	8.38	7.93	8.09

Histopathology results: Histopathological assessment following 7 days post challenge revealed that all the subjects under study were suffering from various histopathological alterations. In control Group (negative); the spleen exhibits normal splenic cords and red pulp sinusoids (Figure 3). The lungs exhibit normal alveolar lining cells (Figure 4). The intestine displays normal villi and intestinal crypts (Figure 5). The liver (g1) displays normal sinusoids (S) and hepatocytes (h) (Figure 6). In control Group (*K. aerogenes* Positive). The spleen exhibits hyperplasia of lymphoid tissue and red pulp congestion (Figure 7). The lungs exhibit severe congestion of alveolar capillaries) along with hyperplasia of pnumocytes type-2 and proliferation of alveolar macrophages (Figure 8). The intestine reveals an active secretion of Paneth cells (Figure 9). Liver exhibits normal organization of hepatic cords along with advanced lobular fibrosis and leukocyte aggregation (Figure 10). In Group 1: The spleen has lymphoid hyperplasia (Figure 11). The lung section shows moderate interstitial thickening with associated inflammatory cells (Figure 12). The intestinal section shows villi thickening with edema and congestion and

marked inflammatory cell infiltrate (Figure 13). The liver section shows normal cord arrangement of liver with minimal pericentral cell swelling of hepatocytes (Figure 14). In Group 2: Section of Spleen: The lymphoid follicles of white pulp have shown marked hypertrophy due to hyperplasia and congestion of sinusoids of red pulp (Figure 15). Section of Lung: The interstitial tissue has shown mild thickening due to inflammatory cells with pulmonary emphysema (Figure 16).

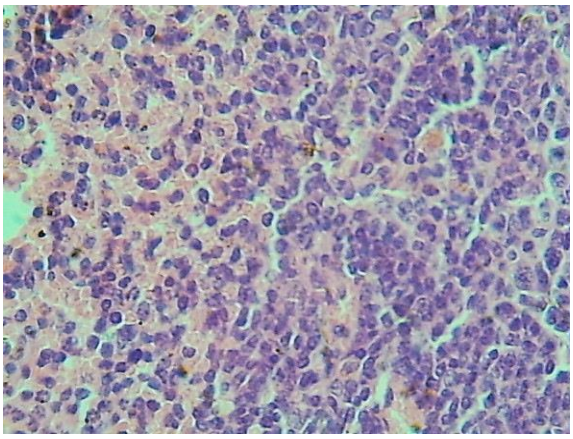

Figure 3. Section of spleen (control negative) shows normal splenic cords & sinusoids of red pulp. H&E.400x

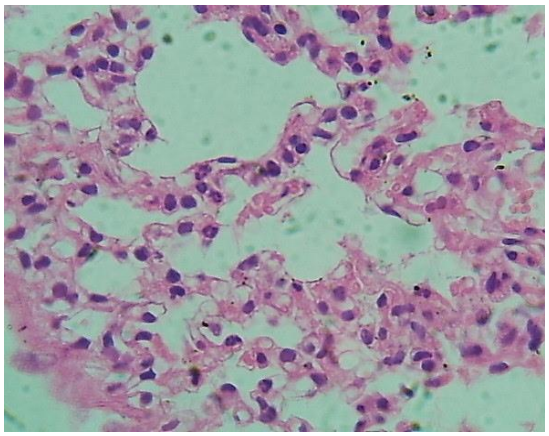

Figure 4. Section of lung (Control negative) shows normal lining cells of alveoli. H&E.400x

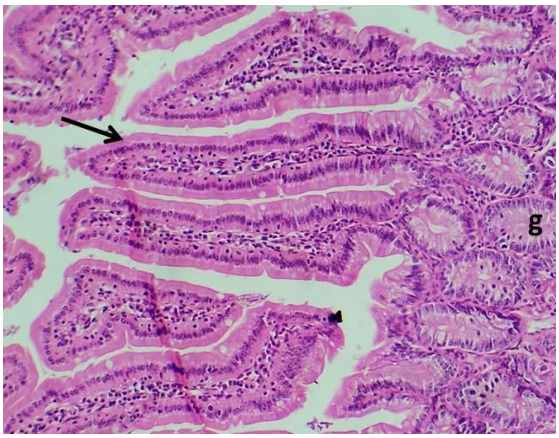

Figure 5. Section of intestine (Control negative) shows normal villi (Arrow), intestinal crypts (g). H&E.100x

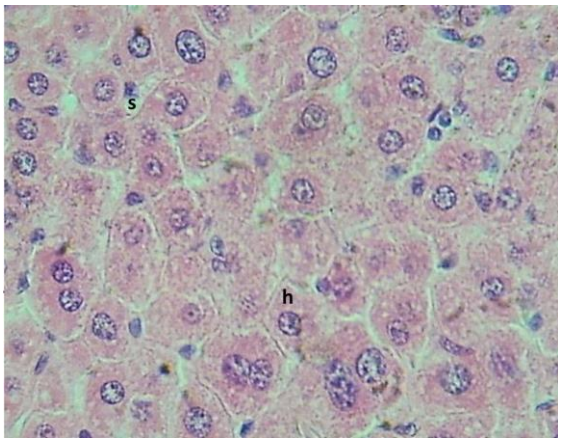

Figure 6. Section of liver (Control negative) shows normal sinusoids (S) hepatocytes (h) .H&E.400x

Section of Intestine: Villi enterocytes, and goblet cells are present normally (Figure 17). Section of Liver: Central veins, hepatocytes, and sinusoids are normal (Figure 18). Group 3: Spleen's section - contains normal lymphoid follicles of the white pulp & sinusoidal congestion & hyperplasia of splenic cords of the red pulp (Figure 19). Lung's section - contains normal bronchioles, normal alveoli & interstitial tissue (Figure 20). Intestine's section - contains normal

enterocytes with normal goblet cells (Figure 21). Liver's section (G3) - contains normal hepatic cords (Figure 22). Control extract only (control E): section of spleen displaying normal lymphoid follicles of the white pulp (black arrow) and red pulp (Figure 23). Section of lungs displaying normal epithelial cells lining the bronchiole and alveoli (Figure 24). Section of intestine showing normal villi and glands of the mucosa (Figure 25). Section of liver displaying normal hepatic cords with minimal vascular changes in hepatocytes and sinusoids (Figure 26).

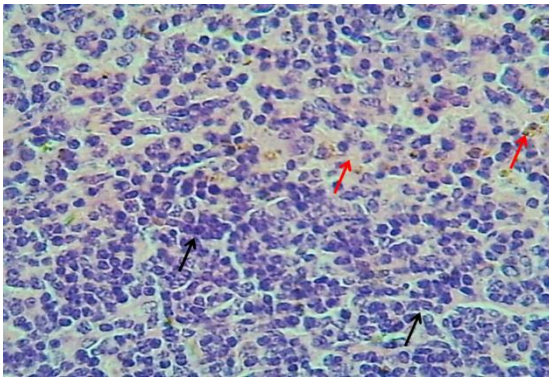

Figure 7. Section of spleen (control positive) shows lymphoid hyperplasia (black arrows) & sinusoidal congestion of red pulp (red arrow). H&E.400x

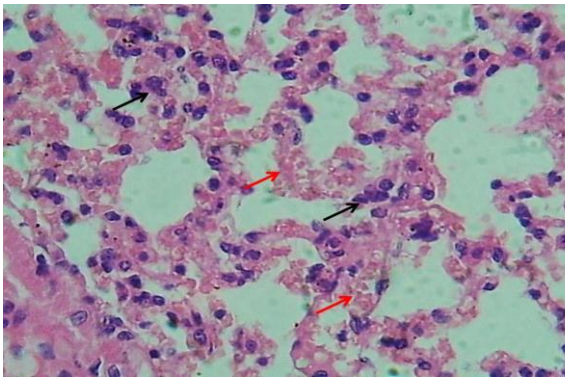

Figure 8. Section of lung (control positive) shows severe congestion of alveolar capillaries (red arrows) & hyperplasia of pnumocytes type-2(black arrows) with proliferation of alveolar macrophages. H&E.400x

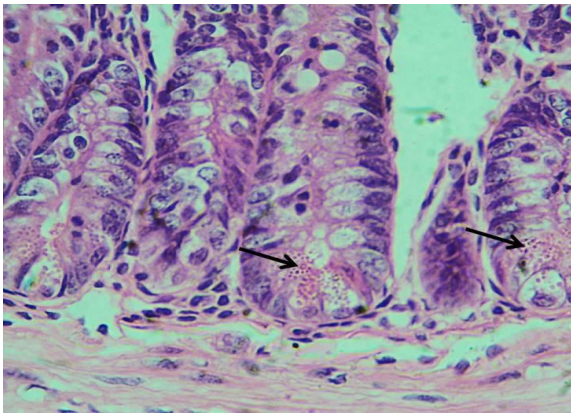

Figure 9. Section of intestine (Control positive) shows active secretory process of Paneth cells. H&E.400x

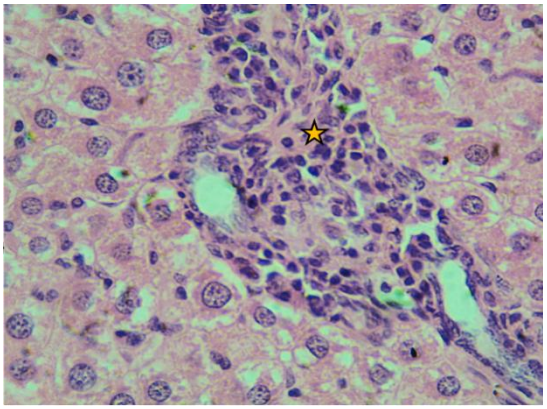

Figure 10. Section of liver (Control positive) shows normal arrangement of hepatic cords with marked advanced lobular fibrosis with marked fibrotic bridge formation and aggregation of leukocytes (Asterisk). H&E.400x

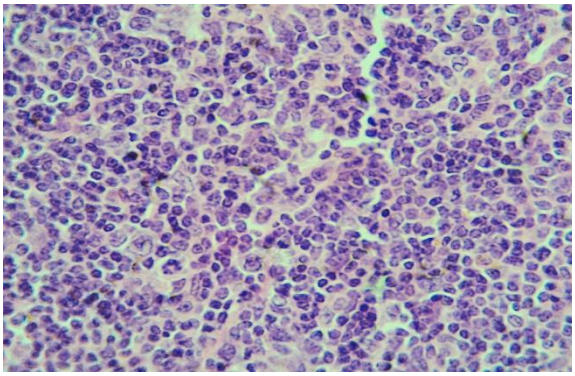

Figure 11. Section of spleen (G1) shows lymphoid hyperplasia (black arrows). H&E.400x

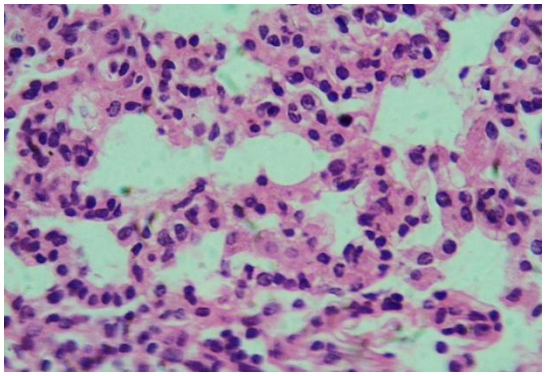

Figure 12. Section of lung (G1) shows mild thickening of interstitial tissue associated inflammatory infiltration. H&E.400x

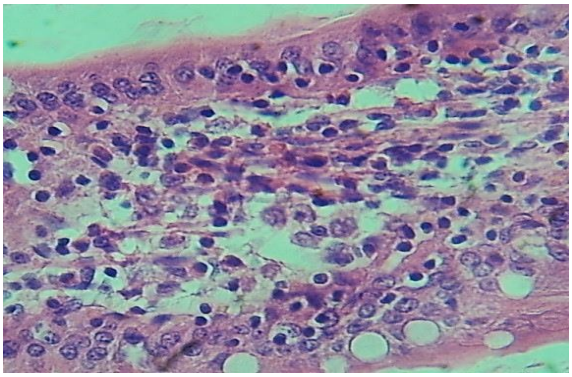

Figure 13. Section of intestine (G1) shows thickening of villi with edema and vascular congestion with profound inflammatory infiltration. H&E.400x

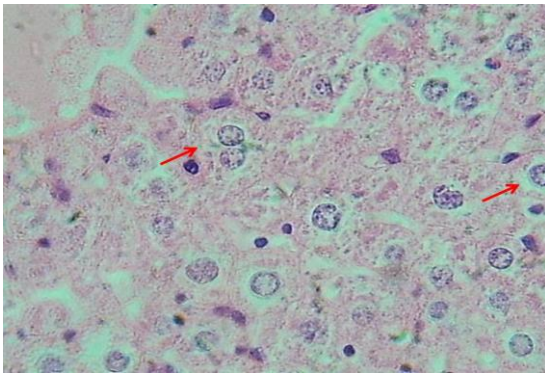

Figure 14. Section of liver (G1) shows normal arrangement of hepatic cords with little mild pericentral cellular swelling of hepatocytes (arrows). H&E.400x

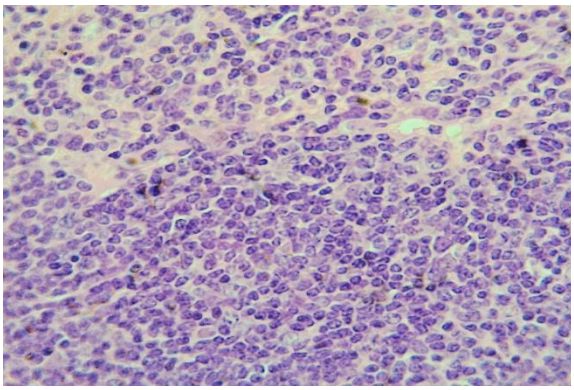

Figure 15. Section of spleen (G2) shows marked hypertrophy of lymphoid follicles of white pulp associated with lymphoid hyperplasia & sinusoidal congestion of red pulp. H&E.400x

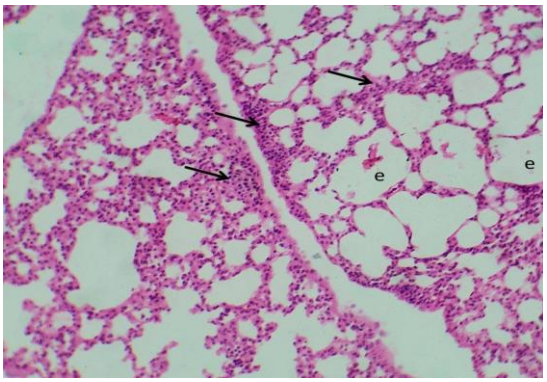

Figure 16. Section of lung (G2) shows mild thickening of interstitial tissue associated with inflammatory infiltration (Black arrows) & pulmonary emphysema (e). H&E.100x

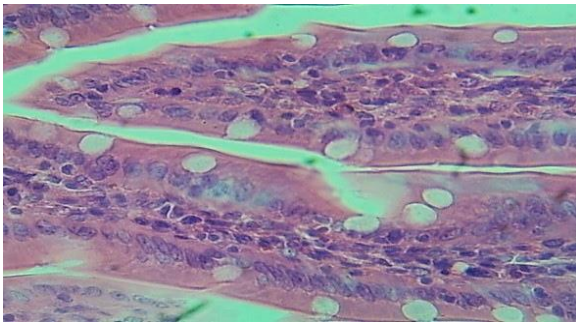

Figure 17. Section of intestine (G2) shows normal villi enterocytes & goblet cells. H&E.400x

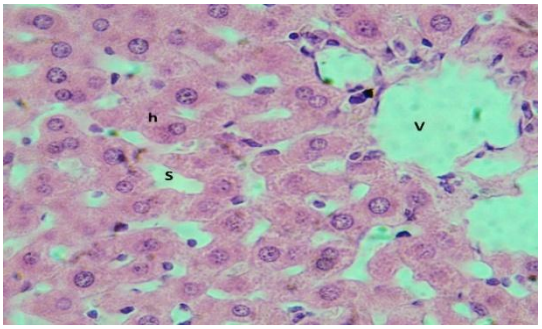

Figure 18. Section of liver (G2) shows normal central vein (v), normal of hepatocyte (h), normal sinusoid (S). H&E.400x

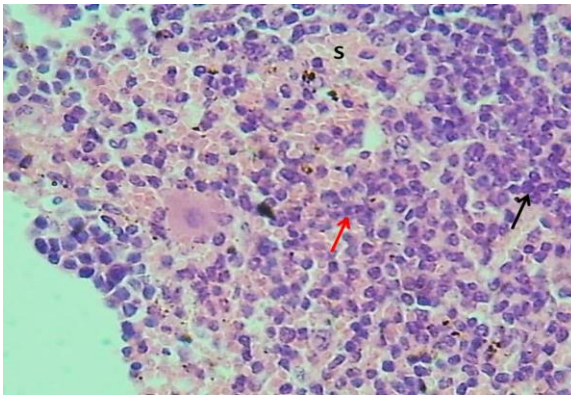

Figure 19. Section of spleen (G3) shows normal lymphoid follicles of white pulp (black arrows) & sinusoidal congestion (S) and hyperplasia of splenic cords of red pulp (red arrow). H&E 400x

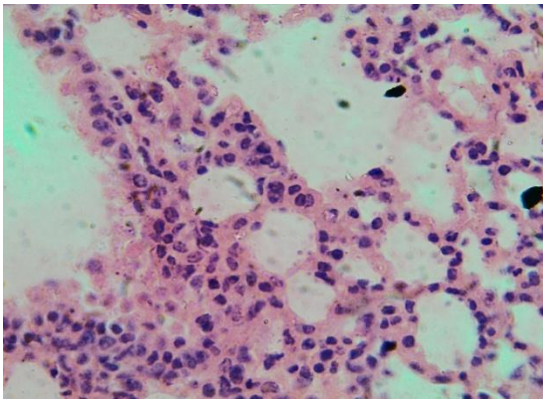

Figure 20. Section of lung (G3) shows normal bronchioles, normal alveoli & interstitial tissue. H&E.400x

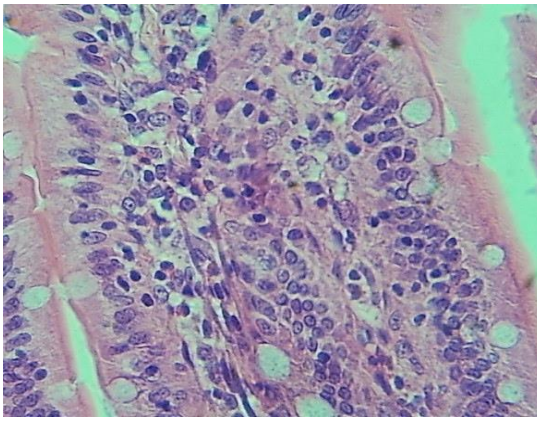

Figure 21. Section of intestine (G3) shows normal enterocytes with normal goblet cells. H&E.400x

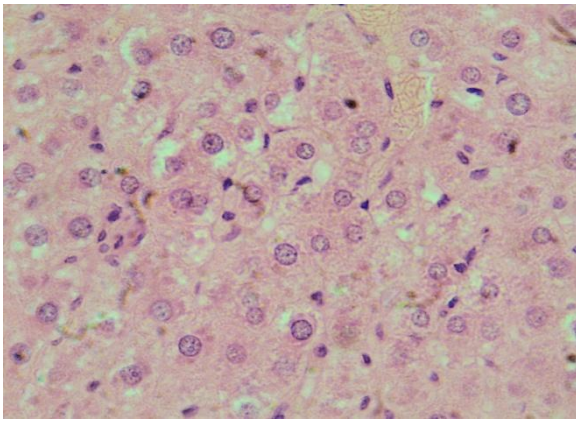

Figure 22. Section of liver (G3) shows normal hepatic cords. H&E.400x

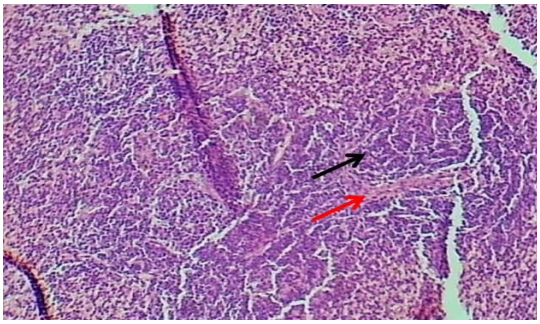

Figure 23. Section of spleen (control E) shows normal lymphoid follicles of white pulp (black arrow) & red pulp (Red arrow).H&E.100x

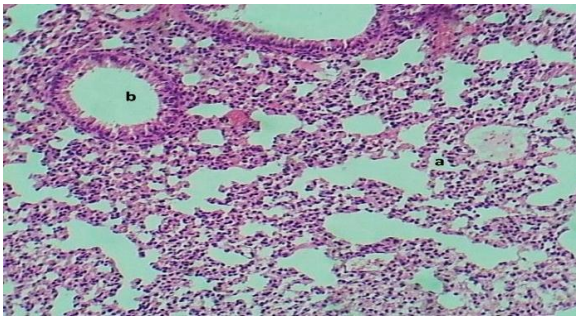

Figure 24. Section of lung (Control E) shows normal lining cells of bronchiole (b) & alveoli (a). H&E.100x

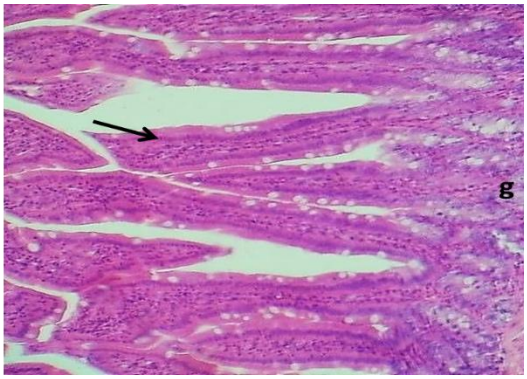

Figure 25. Section of intestine (control E) shows normal mucosal villi & glandular crypts. H&E.100x

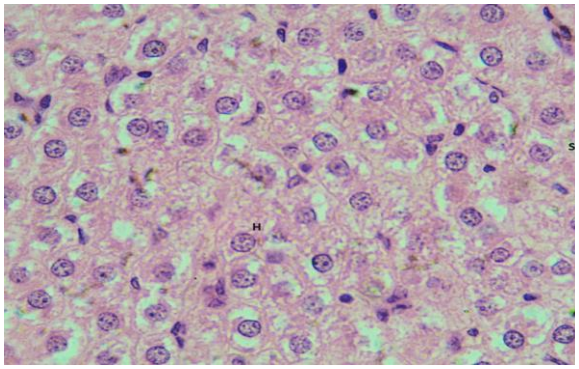

Figure 26. Section of liver (control E) shows normal arrangement of hepatic cords with little vacular changes within hepatocytes (H) & sinusoids (S). H&E.400x

The positive controls (G5) with a value of 2.75 score, denoting serious damage to all organs. However, in negative controls (G4) and extract controls (G6), no lesions were found, resulting in a score of 0. Regarding treatment groups, moderate damage was seen in G1 (250 mg/mL), giving a score of 2.0, whereas mild lesions were recorded for G2 (150 mg/mL), giving a score of 1.0. On the other hand, G3 (50 mg/mL) recorded the minimum score of 0.25. Thus, there was an observed reduction in histopathological damage in the treatment groups, although the reduction was not dose-dependent because the lowest dose produced the best results as seen in (Table 6).

Table 6: Histopathological scoring of examined organs

Group	Spleen	Lung	Intestine	Liver	Overall Score
G1 (250 mg/mL)	2	2	3	1	2.0
G2 (150 mg/mL)	3	1	0	0	1.0
G3 (50 mg/mL)	1	0	0	0	0.25
G6 (Extract control)	0	0	0	0	0
G4 (Control −)	0	0	0	0	0
G5 (Control + K. aerogenes)	3	3	2	3	2.75

Discussion

Klebsiella aerogenes were successfully isolated from feces collected from cats, and hence, domestic animals can be regarded as possible sources of opportunistic and zoonotic pathogens (Martin et al., 2025; Mohammed et al., 2025; Lee et al., 2021). There is previous research carried out in Iraq which showed isolation of *Klebsiella spp*. from pets and their correlation with environment and contact between humans and pets (Zghair et al., 2026). Studies have also shown the isolation of *Klebsiella spp*. in pets around the world, highlighting the significance of pets in the dissemination of antibiotic resistance bacteria (Mihu et al., 2026). The identification of bacterial isolates by VITEK-2 Compact was rapid and accurate in confirming *K. aerogenes*. Previous literature indicated that the use of automated systems in *Enterobacteriaceae* detection is rapid, reliable, and accurate, as evidenced by Iraqi research (Mohammed et al., 2026; Al-Hassan et al., 2024). Thus, the diagnostic procedures applied in this study were scientifically sound and clinically applicable. Regarding immunology, the presence of *K. aerogenes* infection caused a considerable increase in white blood cells (WBC) and IL-6 levels, indicating that the innate immunity had been activated against the infection. This conclusion aligns with the findings of other Iraqi researchers (Ali et al., 2022) and the existing literature on the stimulation of cytokine release during gram-negative bacteria infections. Infections activate LPS-dependent immune pathways, which promote the secretion of IL-6 via NF-κB pathways (Kumar et al., 2020). The use of licorice extract was seen to reduce the changes observed in this case because of the immunomodulatory effect that licorice exerted. Such an effect is due to the presence of active ingredients in the plant like glycyrrhizin and flavonoids that can inhibit the pro-inflammatory cytokines and oxidative stress (Semenescu et al., 2026; Mohammed, 2025). Histologically, there was extensive pathology in the organs like the lungs, liver, and intestine, which included inflammation, congestion, necrosis, and architectural alteration. These are similar findings to those obtained from previous research that focused on *Klebsiella* infections, in which virulence factors from the bacteria like endotoxins and capsules contributed to organ destruction (Huang et al., 2025). On the other hand, treatment with the extract of licorice showed a notable improvement in histology due to reduction in inflammatory infiltration and reversion to normality in tissue architecture. This can be attributed to its anti-inflammatory and antioxidant attributes which minimize cell damage and encourage tissue regeneration (Assar et al*.,* 2021). Conclusively, the results generated from this experiment are in line with those recorded in Iraq and globally since *K. aerogenes* caused major immune and histopathological disorders while licorice showed protection through immunomodulatory activity.

Conclusion: The infection by *Klebsiella aerogenes* resulted in substantial impairment of both immune response as well as tissue architecture as evidenced by an increase in

proinflammatory cytokine levels and histopathological alteration. Treatment with licorice extracts resulted in improvement due to reduced inflammation, regulation of immune response, and proper organization of tissues. It is interesting to note that there was a dose-response relationship whereby the lowest dose was associated with maximum protection. Based on this finding, licorice extract could be used as a partial immunomodulator for bacterial infections.

Author contributions

All the authors have contributed substantially to the paper. Roua J. Mohammed: Idea generation, research design, data acquisition, data analysis, and writing. Ikram Abbas Al Sammaraa: Paper supervision, data validation, and editing of manuscript. Sabrin Ibraheem Mohsin: Data analysis and interpretation, paper editing. All the authors have reviewed and approved the final manuscript.

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Data availability statement

The data contributing to the findings of this study are available from the investigating researcher upon request.

Ethical considerations

Approval was obtained locally by the institutional animal care and use committee at the College of Veterinary Medicine, University of Baghdad (P-G\101,17\2\2026).

Conflicts of interest

There is no conflict of interest among the authors.

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تعدیل طبیعی سیستم ایمنی میزبان در برابر *Klebsiella aerogenes*: شواهدی از عصاره شیرین‌بیان

رؤی ج. محمد

* نویسنده مسئول. گروه میکروبیولوژی، دانشکده دامپزشکی، دانشگاه بغداد، عراق. ایمیل: ruaa.jassem1103a@covm.uobaghdad.edu.iq

اکرام عباس السمراء

گروه میکروبیولوژی، دانشکده دامپزشکی، دانشگاه بغداد، عراق. ایمیل: ikram@covm.uobaghdad.edu.iq

صبرین ابراهیم محسن

گروه میکروبیولوژی، دانشکده دامپزشکی، دانشگاه بغداد، عراق. ایمیل: Sabrin.i@covm.uobaghdad.edu.iq

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چکیده

هدف: ظهور *Klebsiella aerogenes* به‌عنوان یک پاتوژن احتمالی با عفونت‌های گوارشی و سیستمیک در حیوانات و انسان‌ها مرتبط دانسته شده است. همچنین مقاومت ضدمیکروبی در میان سویه‌های گونه‌های *Klebsiella* افزایش یافته است. بنابراین، نیاز به یافتن منابع جایگزین از مواد تعدیل‌کننده سیستم ایمنی وجود دارد. از این‌رو، هدف این مطالعه ارزیابی اثرات تعدیل‌کننده ایمنی و پیشگیرانه عصاره *Glycyrrhiza glabra* (شیرین‌بیان) در مدل موشی عفونت ناشی از *Klebsiella aerogenes* بود.

مواد و روش‌ها: نمونه‌ها از ۵۰ گربه مبتلا به اسهال برای کشت و شناسایی *K. aerogenes* جمع‌آوری شدند. سپس این باکتری‌ها برای ایجاد عفونت تجربی در ۴۸ موش نژاد Swiss albino استفاده شدند. حیوانات به شش گروه شامل هشت موش تقسیم شدند. سه گروه عصاره خوراکی شیرین‌بیان را با دوزهای مختلف ۵۰، ۱۵۰ و ۲۵۰ میلی‌گرم بر میلی‌لیتر دریافت کردند. دو گروه کنترل شامل کنترل منفی (تجویز خوراکی PBS) و کنترل مثبت بودند. گروه اضافی نیز فقط عصاره را دریافت کرد و آلوده نشد. عصاره شیرین‌بیان دو بار در هفته به مدت ۲۱ روز تجویز شد و سپس موش‌ها با باکتری *Klebsiella aerogenes* با غلظت 1.5×10^8 CFU/mL تلقیح شدند.

نتایج: موش‌های آلوده درمان‌نشده در مقایسه با گروه کنترل منفی افزایش معنی‌داری در شاخص‌های التهابی و خونی نشان دادند ($P \leq 0.05$). افزایش قابل توجهی در تعداد گلبول‌های سفید در موش‌های آلوده مشاهده شد، به‌طوری‌که میانگین آن‌ها به $12.8 \pm 1.4 \times 10^3/\mu L$ رسید. در حالی که این مقدار در گروه کنترل $6.2 \pm 0.9 \times 10^3/\mu L$ بود. همچنین، سطح IL-6 در موش‌های آلوده به‌طور معنی‌داری تا 85.6 ± 5.3 pg/mL افزایش یافت، در حالی که در گروه کنترل 28.4 ± 3.1 pg/mL بود. موش‌های آلوده درمان‌نشده دچار ضایعات شدید التهابی و تخریبی بافتی شدند. در مقابل، موش‌های آلوده‌ای که با شیرین‌بیان درمان شده بودند، کاهش التهاب، کاهش سطحIL-6، بهبود شاخص‌های خونی و آسیب بافتی کمتر را نشان دادند. کمترین دوز عصاره (۵۰ میلی‌گرم بر میلی‌لیتر) بیشترین اثر محافظتی را ایجاد کرد که نشان می‌دهد رابطه مستقیمی میان غلظت شیرین‌بیان و اثر زیستی آن در موش‌های آزمایشگاهی وجود ندارد.

نتیجه‌گیری: عصاره *Glycyrrhiza glabra* دارای اثرات ضدالتهابی و محافظتی در برابر عفونت ناشی از *Klebsiella aerogenes* در موش‌ها بود. عصاره شیرین‌بیان موجب کاهش التهاب و بهبود تغییرات پاتولوژیک مشاهده‌شده در حیوانات آلوده شد. بنابراین، *Glycyrrhiza glabra* می‌تواند به‌عنوان یک گزینه امیدوارکننده برای توسعه تعدیل‌کننده طبیعی سیستم ایمنی مطرح شود، هرچند انجام مطالعات بیشتر ضروری است.

کلمات کلیدی: تعدیل سیستم ایمنی، عصاره شیرین‌بیان، IL-6، *Klebsiella aerogenes*

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