Biomedical & Pharmacology Journal, September 2025. Vol. **18**(3), p. 2298-2304

Evaluation of Quercetin on Proinflammatory Cytokines Against *Klebsiella pneumoniae* in Mice

Ikram Abbas Aboud Al Sammaraae

Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq.
*Corresponding author Email: ikram@covm.uobaghdad.edu.iq

https://dx.doi.org/10.13005/bpj/3256

(Received: 31 May 2025; accepted: 08 September 2025)

***Klebsiella pneumoniae* is an opportunistic bacterium associated with severe nosocomial infections, and Multi-drug-resistant represents a considerable risk to public health due to rapidly increasing disease prevalence over the past few decades worldwide .This study aimed to demonstrate the capacity of Quercetin dietary supplement to modulate the immune responses for IL-,[8] IL-,[6] and INF-á. Forty mice use in [4] groups (n=10), the first three groups were received orally 150,100,and 50 mg /mouse weekly for two months, and the 4th group was administered phosphate buffer saline (PBS) as negative control. After two months, sera were collected from mice before and after challenge ([10] days) with an infectious dose of K. pneumoniae (.[15]×108 cfu/ml, I.P) of all groups . The results revealed increased levels of IL-,[6] IL-,[8]and TNF-á significantly (Pd"0.05) at all doses compared to the negative control group and the dose 100mg /ml give the high level at day 60 ,while dose 50mg/ml give the high level after [10] days post infection, but the three treated groups decreased relatively to the negative control after challenge. This research underscores the promise of Quercetin at dose 150mg/ml as anti-inflammatory and immunomodulatory properties through modulation of IL-,[6] IL-,[8] and TNF-á levels, which mitigated excessive inflammation and bolstered host defense mechanisms. The findings indicate that Quercetin may serve as a valuable complementary treatment for infections caused by K. pneumoniae.**

Keyword: *Klebsiella pneumoniae*, Quercetin, ELISA, Mice, Cytokines.

Klebsiella spp. belongs to the family Enterobacteriaceae. It is rod-shaped, facultative anaerobe in shape, Gram-negative, non-motile, capsulated, formic mucoid colonies, that has an affinity to colonize the mucosal surfaces of mammals and human, particularly in oropharynx and gastrointestinal tract, which occurs extensively in various environmental habits such as air, plants, insects, and soil .[1] It represents a significant infectious threat within the poultry sector in their respiratory system which leads to pneumoniae and increase mortality rates or diminish production, particularly in young chicks instances of failure treatment due to the rise of antibiotic-resistant strains ,[1, 2] primarily cause by the misuse of antibiotics facilitates the mutation and development of resistance pathogens .[3] The multidrug-resistant strains poses considerable challenge to the poultry industry's sustainability and public health; Quercetin is demonstrated a strong antimicrobial properties against bacteria, fungi, and viruses and its antimicrobial effects are mainly attributed to

various mechanisms, such as compromising cell membrane integrity, block nucleic acid synthesis, prevent biofilm formation, inducing mitochondrial dysfunction, and reducing the expression of virulence factors; Additionally, the ongoing rise of multidrug-resistant microorganisms has prompted the pursuit of new antimicrobial agents that have a specific action to produce fewer adverse effects .[4] Furthermore, the combination of Quercetin and meropenem reduced the expression of the virulence factors blaVIM and ompC in clinical isolates of E. coli and K. pneumoniae that are part of the carbapenem-resistant Enterobacteriaceae family .[5] The current research aimed at establishing the impact of varied levels of Quercetin on proinflammatory cytokines' level in K. pneumoniae-infected mice.

MATERIALS AND METHODS

Ethical approval

Ethical approval was provided by the college local committee for animals' use and care, College of Veterinary Medicine/ University of Baghdad with number P-G/649, dated 24/3/2024.

Animal of study

Sixty four Swiss mice of both sex between 20-30 g weighted were used ,which propagated and adaptation in the Animal House of Veterinary College / University of Baghdad.

Isolation and identification

Klebsiella pneumoniae was isolated from in thirty poultry fecal samples at Baghdad city. The MacConkey, as well as nutrient agars were inoculated with each sample and incubated at 37 °C for 24hrs. Species identification of the isolates were performed with conventional morphological and biochemical methods and Vitek [2] Compact Systems .[6]

Preparation of Quercetin

Quercetin –Quercetin Dihydrate 500mg capsule (Dietary Supplement),AMAZING NUTRITION , No.22187, USA was prepared in three concentrations (150, 100 and 50 mg/ml) in phosphate buffer saline-PBS.

Cytokines

Concentrations of cytokines (IL-8 IL-6 and INF-á) had been determined by ELISA kits (Elabscience -China + MyBioSource USA) as in the manufacturer's instructions .[7]

Infectious Dose

The infectious dose was standardized by the McFarland tubes technique through three concentrations of bacteria (1.5×10^8 ,3.0×10^8, and 6.0×10^8 CFU/ml) which utilized by using 24 mice that randomly distributed into three equal groups (8 mice/each) infected orally. Clinical manifestations and death were monitored for three days, and the first dose (1.5×10^8 cfu/mL) represented as an infectious dose.

Experimental Animals

Following the pibt study , a total of 40 Swiss mice (20 males and 20 females) were used in the main experiment to evaluate the effect of Quercetin on inflammatory cytokines during *K. pneumoniae* infection separated into four distinct groups, each containing ten mice. The 1st, 2nd, and 3rd groups received Quercetin orally at doses 150, 100, and 50 mg/ml/animal, respectively, once a week for 6 weeks, and the 4th group received orally PBS (1 ml) as negative control. After that all mice were infected intraperitonium I.P by *K. pneumoniae* with infective dose (1.5×10^8 cfu/ml).

Statistical Analysis

SAS (Statistical Analysis System - version 9.1) was used to analyze the data. Two-way ANOVA with interaction and LSD were applied to establish significant differences between means .[8]

RESULTS

Isolation and Identification

From a total of thirty fecal samples collected from poultry, five isolates of *K. pneumoniae* were identified, representing 16.6%. The colonies were seen as pink, mucoid colonies on MacConkey agar (Figure 1)

To verify the identification of the isolates, the Vitek 2 compact system was utilized, yielding a 91% probability for *K. pneumoniae* (Figure 2).

IL-6 concentration

Analysis of IL-6 levels between groups (G1, G2, and G3) shows increase with significant differences (Pd"0.05) on day 60 after Quercetin administration, as observed when compared to day 0 as well as with the negative control group. Group 4 (negative control) has the highest levels of IL-6 at day 70 following infection, with levels of 1577.41 ±41.0 pg/ml. On the other hand, all

treatment groups exhibit a significant reduction in the level of IL-6 compared to the negative control group (Table 1).

IL-8 concentration for mice

The findings relate to IL-8 levels across all experimental groups (G1, G2, and G3) reveal a significant moderate rise at day 60 after the administration of Quercetin with statistically significant (Pd"0.05) when compare to both day 0 and the negative control group. Group 4(negative control) display the highest IL-8 concentrations on day 70 post-infection, with measurements of 1.759 ±0.07ng/ml, while all treated groups show elevated IL-8 levels, which were generally lower than those recorded in the negative control group (Table 2).

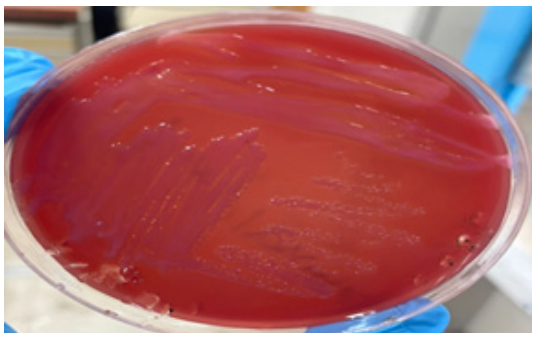

Fig. 1. *Klebsiella pneumoniae* shows pink mucoid colonies on MacConkey agar

TNF-á concentration for mice

The results concerning TNF-á levels across all experimental groups (G1, G2, and G3) indicated a notable moderate rise on day 60 following the administration of Quercetin. This increase was not statistically significant (Pd"0.05) when compared to the measurements taken on day 70 post-infection. In Group 4, which was the positive control, the TNF-á levels were the highest on day 70 after infection, recording values of 526.63 ±22.77 ng/ml. Although all treated groups exhibited higher TNF-á levels, these were typically lower than those observed in the negative control group (Table 3).

DISCUSSION

Klebsiella spp. present in water, soil, and other vegetables and reside in the respiratory and intestinal tracts of man and animals. They can cause airsacculitis in poultry and yolk sac infections in chicks. Klebsiella pneumoniae is a significant pathogen linked to various illnesses, particularly as a secondary infection following viral or environmental issues, especially in birds that affect the respiratory tract .[1,9] The findings of this research indicated that the culture method revealed five isolates out of thirty samples exhibiting

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.83 hours	**Status:** Final
Selected Organism	91% Probability **Bionumber:**	**Klebsiella pneumoniae ssp pneumoniae** 2615734453564610	
ID Analysis Messages			

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	-			

Fig. 2. The Vitek 2 compact system employed for identification of *Klebsiella pneumoniae*

characteristics of Klebsiella spp. colonies. These colonies were capable of growing on McConkey agar and fermenting lactose, resulting in mucoid, bright pink colonies, a distinctive feature of Klebsiella spp. Additionally, biochemical analysis conducted using the VITEK2® Compact system determined that 91% of these bacteria were identified as Klebsiella. pneumoniae.

These findings align with various studies employing conventional methods. The isolation rate in this study was 16.6%, which is consistent with the research conducted by Gorrie et al. [10] on broiler chickens in Al Mansoura, Egypt, as well as the study by Oliveira et al. .[11] This discrepancy may be attributed to regional variations and the differing number of samples analysed in each investigation. Besides, the results of this study are similar to ,[1] which cultured *K. pneumoniae* from Iraqi respiratory diseased chickens, with a diagnosis rate of 30% (15/50), as verified by selective media culture, staining techniques, and biochemical tests, also K. pneumoniae infections cause host cells to secrete various pro-inflammatory cytokines, including IL-1â, IL-6, IL-8, TNF-á, and IL-17.

These cytokines are important in recruiting and activating the immune cells, which in turn strengthens the defense of the host against bacterial

Table 1. level of IL-6 in the group administered various doses of Quercetin, as determined by the ELISA assay

Days/ Groups	Zero day	Mean ± SE (ng /ml) At day 60 before infection	At day 70 after infection	L.S.D. value
G1: Qurecetin (150 mg/ml)	34.98 ±0.97 AB c	287.53 ±13.75A b	560.80 ±14.13C a	34.363 *
G2: Quercetin (100 mg/ml)	35.37 ±0.54 A c	302.11 ±14.68A b	525.86 ±21.54C a	45.386 *
G3: Quercetin (50 mg/ml)	31.43 ±2.15 BC c	215.54 ±10.25B b	800.90 ±32.45B a	59.349 *
G4 (negative control) PBS	30.93 ±0.62 C b	32.39 ±1.16C b	1577.41 ±41.0A a	71.391 *
L.S.D. value	3.697 *	33.35 *	85.985 *	—-

Different big letters in the same column and small letters in the same row mean they differs significantly. * (P≤0.05).

Table 2. Level of IL-8 in the group administered various doses of Quercetin, as determined by the ELISA assay

Days/ Groups	Zero day	Mean ±SE (ng /ml) At day 60 before infection	At day 70 after infection	L.S.D. value
G1:Qurecetin (150 mg/ml)	0.134 ±0.01 AB c	0.427 ±0.03A b	0.827 ±0.02B a	0.061 *
G2: Quercetin (100 mg/ml)	0.135 ±0.01A b	0.401 ±0.03A b	0.908 ±0.04 B a	0.089 *
G3: Quercetin (50 mg/ml)	0.131 ±0.01BC c	0.264 ±0.01B b	0.901 ±0.02B a	0.037 *
G4 (negative control) PBS	0.130 ±0.01C b	0.132 ±0.01C b	1.759 ±0.07A a	0.126 *
L.S.D. value	0.0037 **	0.070 *	0.126 *	—-

Different uppercase letters within the same column and lowercase letters across the same row indicate a significant difference. * (Pd”0.05)

Table 3. level of TNF-á in the group administered various doses of Quercetin, as determined by the ELISA assay

Days/ Groups	Mean ±SE (ng /ml) Zero day	Day 60 before infection	Day 70 after infection	L.S.D. value
G1:Qurecetin (150 mg\ml)	17.36 ±1.26A c	65.16 ±1.09A b	109.76 ±4.17C a	7.815 *
G2: Quercetin (100 mg/ml)	12.84 ±0.62B c	59.04 ±0.71B b	98.96 ±3.17C a	5.760 *
G3: Quercetin (50 mg/ml)	14.78 ±0.66B c	36.13 ±2.04C b	262.94 ±5.10B a	9.365 *
G4 (negative control) PBS	14.03 ±0.65B b	13.67 ±0.85D b	526.63 ±22.77A a	39.672 *
L.S.D. value	2.498 *	3.787 *	35.276 *	—-

Different uppercase letters within the same column and lowercase letters across the same row indicate a significant difference. (P≤0.05)

infections, the dissemination of multidrug-resistant and highly virulent *K. pneumoniae* strains represents a significant obstacle in the effective treatment of such infections, and discuss alternative strategies to combat these severe infections .[12,13]Quercetin is acknowledged as a significant natural compound, distinguished by its strong ability to influence immune system functions and its anti-inflammatory properties .[14,15] In this research, we have examined the immunomodulatory potential of Quercetin and its anti-inflammatory action against *K. pneumoniae* infections, with particular focus on the production of pro-inflammatory cytokines. The findings based on our study showed that at 60 days after the administration of Quercetin, IL-6, IL-8, and TNF-á levels were significantly higher (P<0.05) in all groups receiving varying doses compared to the negative control. Moreover, on day 7 following infection with the infectious dose of *K. pneumoniae*, the concentrations of the aforementioned cytokines also showed a significant increase (P<0.05) in all groups relative to the negative control. Conversely, relative to the positive control, the concentrations of IL-6, IL-8, and TNF-á showed a significant decrease (P<0.05) on day 7 following the infection. These data suggest the anti-inflammatory and immunomodulatory activities of Quercetin, which point to flavonoid-mediated immunomodulatory effect target potential. They also suggest the clinical relevance of these flavonoid effects in K. pneumoniae infections. Its infection induces host cells to produce various pro-inflammatory cytokines including IL-1â, IL-6, IL-8, TNF-á, and IL-17. The findings in this study validate previous research ,[16] indicating that Quercetin can be employed as a prophylaxis. In this article, it talks about the immunotoxicity and oxidative stress induced by LPS. The toxicity was reduced with the administration of Quercetin, as was shown with reduced IL-33 and TNF-á levels, along with raised antioxidant levels such as GSH and CAT, and demonstrating anti-cancer activity. Therefore, Quercetin may be a promising therapeutic agent. Such cytokines play critical role in activating and mobilizing immune cells, optimally increasing the host's capacity to fight bacterial infections.

CONCLUSION

This research identifies the therapeutic potential of Quercetin as an anti-inflammatory and immunomodulatory drug in Klebsiella pneumoniae infections. Quercetin therapy was able to enhanced the immune response by modulating IL-6, IL-8, and TNF-á levels that alleviated excessive inflammation and improved host defense mechanisms. The findings indicate that Quercetin may be used as an effective complementary drug for infections caused by *K. pneumoniae.*

ACKNOWLEDGEMENT

The author would like to thank Baghdad university \College of Veterinary medicine for

granting the research work. The Department of Microbiology\College of Veterinary medicine, is highly appreciated for allowing the bacterial isolation and ELISA technique in laboratory work.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

Ethical approval was provided by the college local committee for animals' use and care, College of Veterinary Medicine/ University of Baghdad with number P-G/649, dated 24/3/2024

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required

Clinical Trial Registration

This research does not involve any clinical trials

Permission to reproduce material from other sources

Not Applicable

Author Contributions

The sole author was responsible for the conceptualization, methodology, data collection, analysis, writing, and final approval of the manuscript.

REFERENCES

1. Al-AAlim AM, Al-Chalaby AY, Al-Abedi SF. Isolation and identification of Klebsiella pneumoniae from respiratory disease in chicken. *Iraqi Journal of Veterinary Sciences*. 2024; 30;383:583–8 .
2. Hamza E, Dorgham SM, Hamza DA. Carbapenemase-producing Klebsiella pneumoniae in broiler poultry farming in Egypt. *Journal of Global Antimicrobial Resistance*. 2016; 27;7:8–10.
3. Khudhir ZS. Evaluation the Antibacterial Activity of the Brine, Nisin Solution, and Ozonated Water Against E. coli O157:H7 in the Experimentally Local Produced Soft Cheese. *The Iraqi Journal of Veterinary Medicine*. 2021; 28;451:17–21.
4. Nguyen TLA, Bhattacharya D. Antimicrobial activity of Quercetin: An approach to its mechanistic principle. *Molecules* . 2022; 12;278 -2494.
5. Pal A, Tripathi A. Demonstration of bactericidal and synergistic activity of Quercetin with meropenem among pathogenic carbapenem resistant Escherichia coli and Klebsiella pneumoniae. *Microbial Pathogenesis*. 2020; 10;143-104120.
6. Hadi SM, Aldujaili NH. Bio-Environmental preparation of Selenium Nanoparticle using Klebsiella Pneumoniae and their Biomedical Activity. *IOP Conference Series Earth and Environmental Science* .2022; 1;10291-012021.
7. Mohammed RJ, Al-Samarraae IAA. Investigating the Effect of Three Antigens of Citrobacter freundii on Rabbit's Immune Response. *The Iraqi Journal of Veterinary Medicine* .2021;28;451:56–62.
8. SAS/STAT. Users Guide for Personal Computer. Release 9. 1. SAS Institute, Inc., Cary, N.C., USA.2010
9. Al-Juburi LI, Al-Sammarraae IA. The Protective Role of Salmonella Typhimurium-Whole Sonicated Killed Antigen and Syzygium aromaticum Extract on the Histopathological Changes Against Its Infection in Rabbits. *The Iraqi Journal of Veterinary Medicine* . 2022 ; 28;462:12–9 .
10. Daehre K, Projahn M, Friese A, Semmler T, Guenther S, Roesler UH. ESBL-Producing Klebsiella pneumoniae in the Broiler Production Chain and the First Description of ST3128. *Frontiers in Microbiology* . 2018;3;9.
11. Oliveira R, Castro J, Silva S, Oliveira H, Saavedra MJ, Azevedo NF, et al. Exploring the Antibiotic Resistance Profile of Clinical Klebsiella pneumoniae Isolates in Portugal. *Antibiotics* . 2022;13;1111-1613.
12. Gorrie CL, Mirèeta M, Wick RR, Judd LM, Lam MMC, Gomi R, et al. Genomic dissection of Klebsiella pneumoniae infections in hospital patients reveals insights into an opportunistic pathogen. *Nature Communications* . 2022;31;131.
13. Wei S, Xu T, Chen Y, Zhou K. Autophagy, cell death, and cytokines in K. pneumoniae infection: therapeutic perspectives. *Emerging Microbes & Infections* . 2022;26;121.
14. Tanaka T, Hirano T, Kawai M, et al. Flavonoids and related compounds as anti-allergic substances. *World Allergy Organization Journal*.

2007; NA;S148.

15. AkheelMM, Al-Mnaser AA, Quijada J, Woodward, Rymer C.Use of tannin-containing plants as antimicrobials influencing the animal health. *Iraqi J. Vet.* 2021; 452:33-40.

16. Al-Kubaisi ZA, Al-Shmgani HS, Salman MJ. Evaluation of In vivo and In vitro protective effects of Quercetin on lipopolysaccharide-induced inflammation and cytotoxicology. *Research Journal of Pharmacy and Technology* . 2020;1;138-3897.