Histopathological alterations caused by Klebsiella pneumoniae infection in rabbits and the preventive effects of whole sonicated killed antigen and Albizia lebbeck extract

By Adian A. Dakl

WORD COUNT 3134
TIME SUBMITTED 25-APR-2024 12:30PM
PAPER ID 108526779

Histopathological alterations caused by *Klebsiella pneumoniae* infection in rabbits and the preventive effects of whole sonicated killed antigen and *Albizia lebbeck* extract

Adian A. Dakl*, Ikram A. Al-Samrraee

Department of Microbiology, Veterinary Medicine College ,University of Baghdad, Iraq

*corresponding:

Email: adian.abd@mu.edu.iq

ABSTRACT

Background. The purpose of this study is to estimate the protective capability of ethanolic *Albizia lebbeck* leaf extract against *Klebsiella pneumoniae* contagion in rabbits.

Methods. The animals gathered into three groups each group contain eight albino rabbits were received the following: Subcutaneously group one was received Killed Whole Cell Sonicated Antigen-Klebsiella pneumoniae (KWCSAg-KP); group two was orally received ethanolic *Albizia lebbeck* leaf extract and KWCSAg-KP subcutaneously; group three was quit as control group was given PBS subcutaneously. The rabbit groups were orally challenged by 1.5 x10^8 CFU/ml Klebsiella pneumoniae then the rabbit were immolated ten days post-challenge for pathological changes. Spleen, liver ,intestine and kidney were examined for histopathological alterations. Intestine, liver, kidney and spleen were examined for pathological changes.

Results. The results revealed that the 2nd group had the least histopathological alterations with moderate infiltration of inflammatory cells in addition to no any abnormality detected in the kidney as well as the liver. The KWCSAg-KP group revealed sloughing with mild superficial inflammation in the lamina propria and mild vacuolation of the hepatocytes in the centrilobular area in addition to moderate white pulp hyperplasia in the spleen .The control group showed sever changes of inflammation, vacuolation, intestinal hemorrhage and congestion of the hepatic veins as well as hemosiderosis of the red pulp in the spleen.

Conclusion. *Albizia lebbeck* leaf extract revealed to give an appropriate protection against inflammation as well as damage of virulent *Klebsiella pneumoniae* contagion in comparison to both of KWCSAg-KP and control groups.

Key word: *Albizia lebbeck*, histopathology, *Klebsiella pneumoniae*

Introduction

Klebsiella is an influential microorganism posing a threat to public health due to its transmission as animal-borne bacterial pathogens (zoonosis) [1].These microorganisms are responsible for a wide range of infections in both humans and animals. These infections can be caused by a variety of initiators, including nosocomial contact, direct or indirect contact with animals that have been infected, and contaminated dairy products [2]. Some of the infections that can be caused by these microorganisms include acute injuries to organs with mucous membranes and infected wounds, bacteremia, meninges-related infections, and abscesses that are located in different parts of the body [2,3]. *Klebsiella*, on the other hand, has been identified from respiratory infections in a variety of domestic animals, including sheep [4] and has the potential to induce sepsis in lambs [5]. Furthermore, it's an essential pulmonary bacteria for camels, calves, horses, foals, sows and horses. It has been linked to a wide range of animal diseases, including a number of systemic infections,

as well as diarrhea in calves, mastitis in cattle, metritis in mares, and meningitis in piglets [6]. An investigation into the pathological changes that are brought about by an infection with *Klebsiella pneumoniae* in the urinary organs is conducted by Ibrahim (2008) [7]. The resistance character of this bacteria it's belong to a complex structures as antigenic determinants include capsular polysaccharides (CPS) and lipopolysaccharides, enhancing main self-protection against environment, effect of disinfectants, many antibiotics, serum factors as well as multiple forms of capsular K antigens cause different levels of infection severity through phagocytic killing action [8,9], that explain how *Klebsiella* promote fatality . Further virulence factors include endotoxin generation, iron acquisition systems as well as adhesins [10].

Several strategies has been followed to evading defense of immune system. *Klebsiella* hidden techniques are shown by capsular polysaccharides functions in suppressing inflammatory response activation, blocking complement's bactericidal impact through Lipopolysaccharides' function in reducing complement deposition at the surface of the bacteria furthermore the absence porins production to prevent complement activation and therefore eliminating phagocytosis by immune cells. On the other hand modification its lipid A intermediate structure between the highly antagonistic hexa-acylated and less antagonistic hepta-acylated versions to halt the protracted and excessive immune activation [11].

The purpose of this study is to assessment the protective capability of ethanolic *Albizia lebbeck* leaf extract against *Klebsiella pneumoniae* contagion in rabbits.

Material and Methods

Ethics

Both the experimental design and the procedures that were utilized in this study were examined and authorized by the Scientific Committee of the Department of Microbiology, College of Veterinary Medicine, University of Baghdad. This was done in compliance with the ethical principles that are concerning animal welfare (Approval Number 2317 / 2023).

Killed Whole Cell Sonicated Antigen- Klebsiella pneumoniae preparation

The isolate *Klebsiella pneumoniae* was identification and characterized using standard and molecular methods [12,13] at Microbiology department, Veterinary medicine collage,

Baghdad University, on ordinary and HiChrom media to prepare antigen. The Killed Whole-Cell Sonicated Antigen of *Klebsiella pneumoniae* (KWCSA-KP) has been produced in accordance with the procedure described in the references [14,15].

Experiment animals

There were twenty-four Albino rabbits, both male and female, that were obtained from the animal house of the College of Veterinary Medicine at the University of Baghdad. The rabbits ranged in age from six months to twelve months and weighed between one thousand and one thousand and five hundred grams. The animals were housed under stander circumstances for controlled measurements.

Experimental Design

The rabbits were gathered into three groups of eight at random. Group one received a subcutaneous (S/C) injection of 1 mL of KWCSAg-KP at a dosage of 1000 µg/mL. Group two received an oral dose of *Albizia lebbeck* leaf extract (300 mg/kg) on alternate days for one week before to the first day of the experiment, and subcutaneously, they were immunized with 1 mL of KWCSAg-KP at a dosage of 1000 µg/mL. Group three , which served as a control, 1 ml of PBS was inoculated subcutaneously. Both the first and second groups of KWCSAg-KP S/C recipients got their booster doses on the fourteenth day after the initial injection. In contrast, the second group received an extra dosage of *Albizia lebbeck* extract orally equal to 300 mg/kg. On day 21, all groups of rabbits were orally administered the challenge dosage of pathogenic *Klebsiella pneumoniae*, which was (1.5 x 10^8 CFU/ml) [16]. Organs such as the liver, spleen, kidney, and intestine were taken out for histological examination when the animals were euthanized ten days after the challenge dose, in accordance with [17].

Results

Histological alterations

Intestine

The first group reveals few areas of superficial epithelial sloughing with mild superficial inflammation in the lamina propria (Figure 1),the second group shows normal epithelium with mild infiltration of inflammatory cells (Figure 2) .Third group shows hyperplasia of the mucosal epithelium with inflammation (Figure 3).

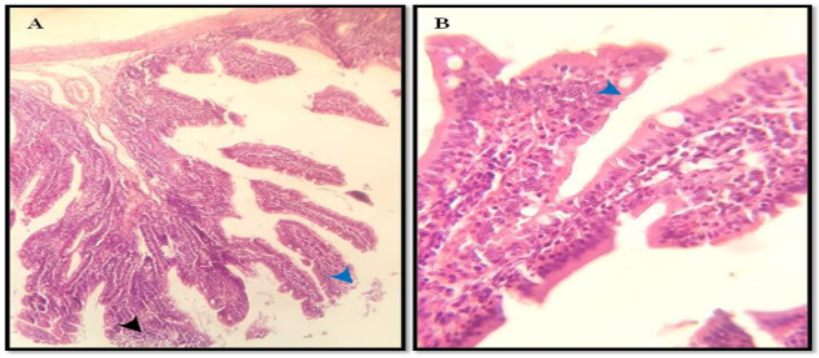

Figure 1. Histopathological sections in the small intestine of adult rabbits immunized with 1000µg/ml of killed whole cell sonicated Antigen- *Klebsiella pneumoniae* (KWCSAg-KP) revealing mild inflammation in the superficial portion of the villi (black arrow), area of epithelial sloughing (blue arrow) H&E A(10X B(40X.

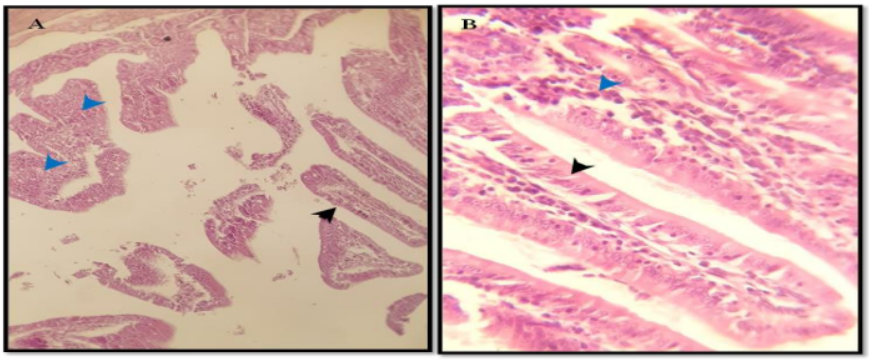

Figure 2. Histopathological sections in the small intestine of adult rabbits administered *Albizia lebbeck* leaf extract at a dose of 300 mg/kg and immunized with 1000µg/ml of killed whole cell sonicated Antigen- *Klebsiella pneumoniae* (KWCSAg-KP) revealing normal epithelium of the intestinal mucosa (back arrow), mild inflammatory infiltration in the lamina propria (blue arrow) H&E A(10X B (40X.

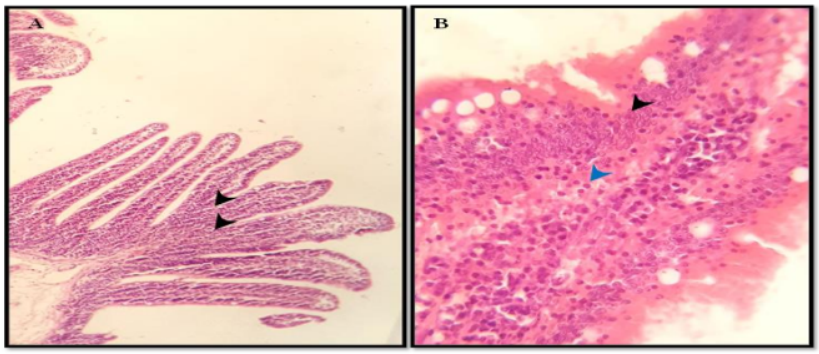

Figure 3. Histopathological sections in the small intestine of adult rabbits of positive control group (inoculated 1 ml PBS) revealing hyperplasia of epithelium of the intestinal mucosa (black arrow) inflammatory, inflammation in the lamina propria (blue arrow) H&E A(10X B (40X

Liver

Liver in the first group shows mild vacuolation of the hepatocytes in the centrilobular area otherwise no any abnormality was detected (Figure 4). Hepatocytes in the liver of the second group appear normal as the other components in both centrilobular and portal area (Figures 5). Third group shows liver there was congestion of the hepatic veins in the centrilobular and portal area (Figures 6). with bridging inflammation extend between central and portal area (Figures 7).

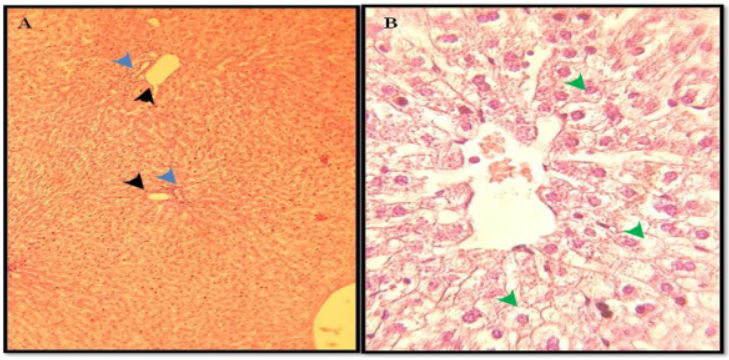

Figure 4. Histopathological sections in the liver of adult rabbits immunized with 1000μg/ml of killed whole cell sonicated Antigen- *Klebsiella pneumoniae* (KWCSAg-KP) revealing normal liver architecture in the portal area; including portal vein (black arrow), bile canaliculi (blue arrow), mild vacuolation of hepatocytes in the centrilobular area (green arrow) H&E A(10X B) 40X

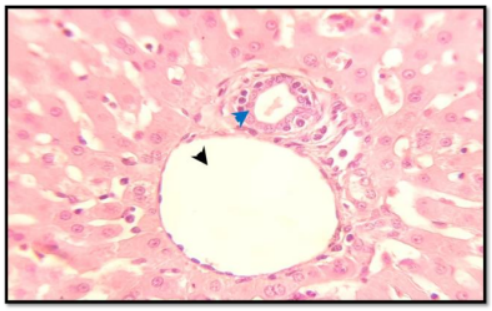

Figure 5. Histopathological section in the liver of adult rabbits administered *Albizia lebbeck* leaf extract at a dose of 300 mg/kg and immunized with 1000μg/ml of killed whole cell sonicated Antigen-*Klebsiella pneumoniae* (KWCSAg-KP) revealing normal liver architecture in the portal area; including portal vein (black arrow), bile canaliculi (blue arrow) H&E 40X.

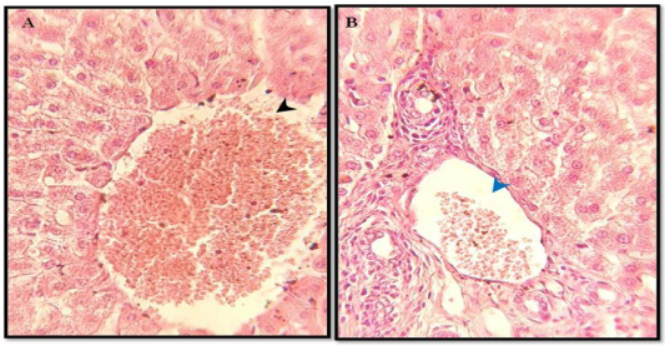

Figure 6. Histopathological sections in the liver of adult rabbits of positive control group (inoculated 1 ml PBS) revealing central vein congestion (back arrow), mild congestion of portal vein (blue arrow). H&E A) & B) 40X.

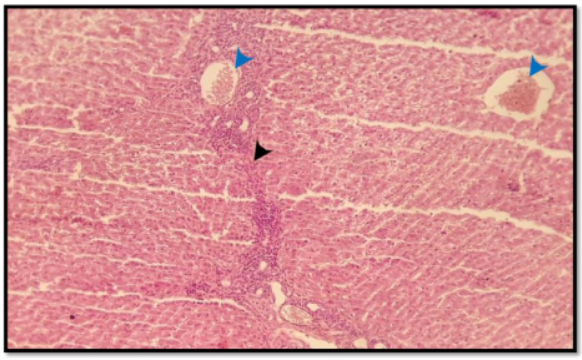

Figure 7. Histopathological section in the liver of adult rabbits of positive control group (inoculated 1 ml PBS) revealing bridge inflammation (back arrow) and vascular congestion (blue arrow). H&E 10X

Kidney

Histopathological inspection of the organs in the present study showed that kidney in the group 1 appears within normal limits, the glomeruli were normal, renal tubular epithelium were consist of single layer of cuboidal cells in both cortex and medulla(Figure 8). In group two there was no any abnormality detected in the kidney, as the glomeruli appear of normal architecture as well as the other renal components that include renal intestitium, vasculature and the renal tubules in both cortex and medulla(Figure 9). Group three shows sever changes in the kidney; these are include, marked glomerular atrophy with vacuolation of renal tubular epithelium in cortex and medulla(Figure 10), marked intestinal hemorrhage and inflammation (Figure 11).

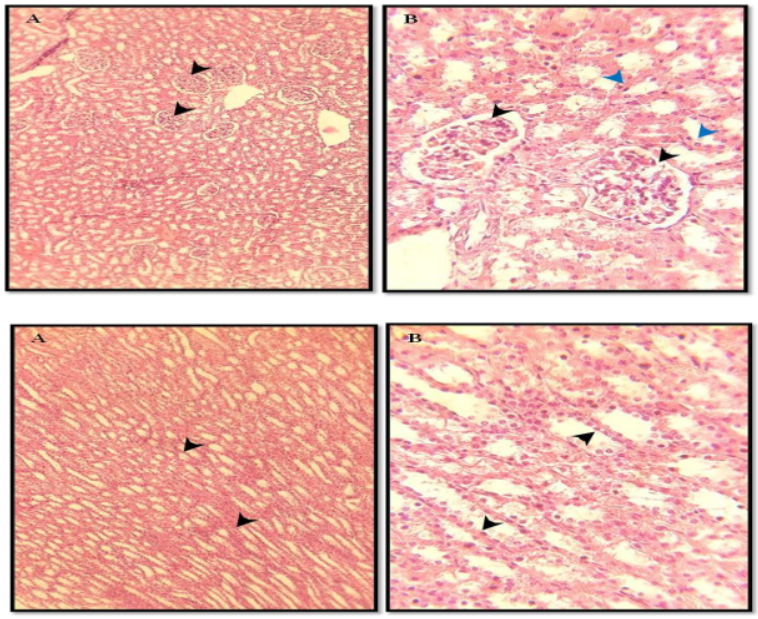

Figure 8. Histopathological sections in the kidney of adult rabbits immunized with 1000µg/ml of killed whole cell sonicated Antigen- *Klebsiella pneumoniae* (KWCSAg-KP) revealing normal glomeruli (black arrow) normal renal tubular epithelium in the renal cortex (blue arrow) A) 10X B) 40X and in the medulla (black arrow) H&E A)10X B) 40X.

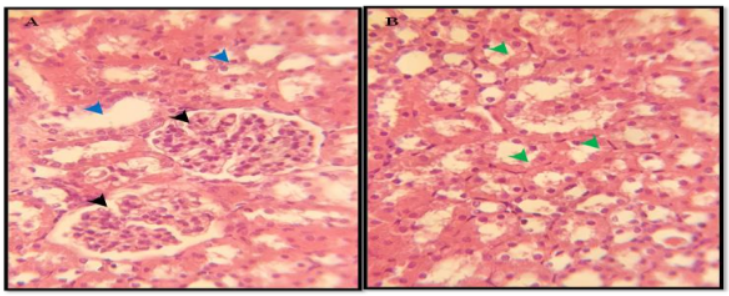

Figure 9 .Histopathological sections in the kidney of adult rabbits administered *Albizia lebbeck* leaf extract at a dose of 300 mg/kg and immunized with 1000µg/ml of killed whole cell sonicated Antigen-*Klebsiella pneumoniae* (KWCSAg-KP) revealing normal glomeruli (black arrow), normal tubules both cortex (blue arrow) and medulla (green arrow). H&E A)&B) 40X.

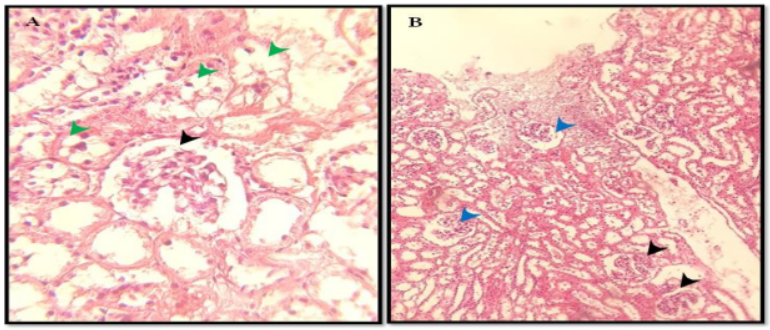

Figure 10 . Histopathological section in the kidney of adult rabbits of positive control group (inoculated 1 ml PBS) showing normal glomerulus (black arrow), some other atrophied (blue arrow), mild vacuolation renal tubular epithelium (green arrow) H&E A) 40X B)10X.

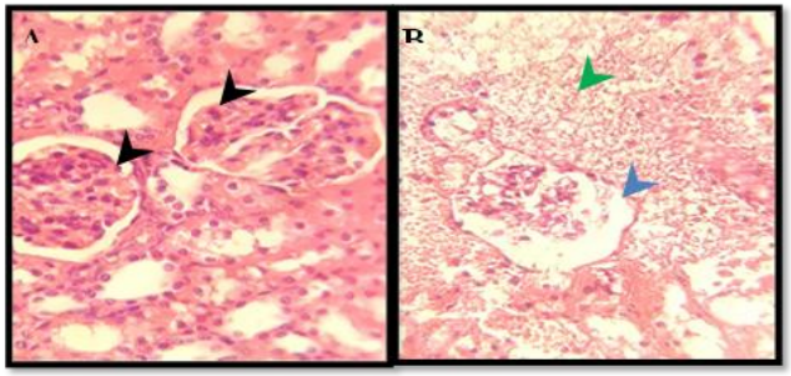

Figure 11 . Histopathological section in the kidney of adult rabbits of positive control group (inoculated 1 ml PBS) showing mild glomerular congestion (black arrow), atrophic glomerulus (blue arrow), interstitial hemorrhage (green arrow) . H&E A) & B) 40X

Spleen

In the first group spleen shows moderate white pulp hyperplasia with normal red pulp (Figure12).In the second group spleen shows mild white pulp hyperplasia with normal red pulp (Figure 13) .In the third group shows spleen hyprplasia of white pulp was sever with hemosiderosis of the red pulp (Figure 14).

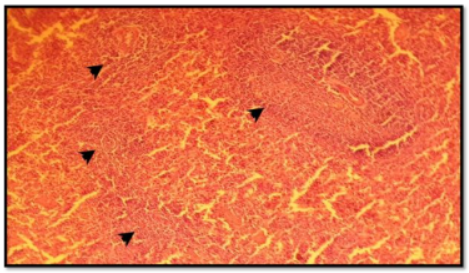

Figure 12. Histopathological section in the spleen of adult rabbits immunized with 1000µg/ml of killed whole cell sonicated Antigen- *Klebsiella pneumoniae* (KWCSAg-KP) revealing moderate white pulp hyperplasia with normal red pulp H&E.

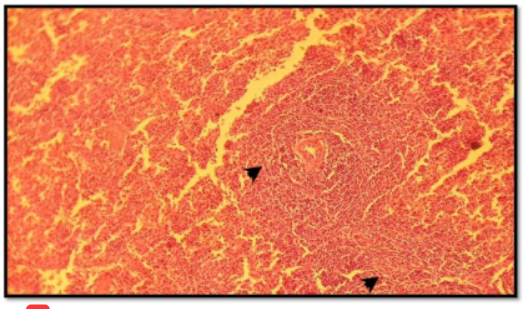

Figure 13. Histopathological section in the spleen of adult rabbits administered *Albizia lebbeck* leaf extract at a dose of 300 mg/kg and immunized with 1000µg/ml of killed whole cell sonicated Antigen- *Klebsiella pneumoniae* (KWCSAg-KP) revealing hyperplasia of white pulp (black arrow) H&E 10X .

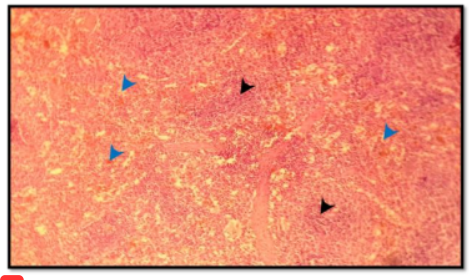

Figure 14. Histopathological section in the spleen of adult rabbits of positive control group (inoculated 1 ml PBS) showing spleen hyprplasia of white pulp was sever with hemosiderosis of the red pulp.

Discussion

The purpose of this research was to determine whether or not the immunomodulatory *Albizia lebbeck* leaf extract and the killed sonicated *Klebsiella pneumoniae* antigen could prevent *Klebsiella pneumoniae* infections.

This study's histopathology results provided more evidence that the *Klebsiella pneumoniae* vaccine provided some protection against the disease that caused by *Klebsiella spp* . The first group showed less pathological results compared to the control group, whereas the second group demonstrated less pathological alterations and greater protection against the viable challenge dosage when injected *Klebsiella pneumoniae* antigen with Albizia lebbeck leaf extract. This demonstrated that the *Albizia lebbeck* leaf extract as anti-inflammatory and preventive capabilities is best than *Klebsiella pneumoniae* antigen alone. In contrast to the moderate inflammatory response shown in the first group treated with the *Klebsiella pneumoniae* antigen, the control group exhibited a wide array of pathological alterations. Because of the high level of protection against the infection, the second group likewise showed the fewest pathogenic alterations.

Other research has debated the importance of synergic action of herbal extract (*Syzygium aromaticum*) combined with killed antigen of (*Salmonella Typhimurium*) in immune modulation and the ability immune response in determining the histopathological changes [18]. These findings are in alignment with previous research that shown pathological symptoms of early infection in mice injected with killed antigen. Specifically, the animals revealed "polymorphonuclear leukocytes in the organs of the reticuloendothelial system", which is indicative of an infection. Vaccinated animals, in contrast to control mice, exhibited mostly small, self-limiting lesions [19].

Humad *et al.*, 2020 also describe in a study the limitation of histopathological alterations of vaccinated mice with *K. pneumoniae* as very low pathological alterations causing no disseminated of infection with a comparatively small vaccine [16]. On the other hand other study revealed inhibition of capsular (k-antigen)for severs histological changes in the intestine following parasitic infection compared with positive control [20].

`According to the kind and mode of infection , bacteria of *Klebsiella spp.* may attached and offensive epithelial cells of the upper respiratory tract , cells in gastrointestinal tract, endothelial cells, or uroepithelial cells, followed by colonization of mucosal membranes [21]. Epidemiologic researches demonstrate that gastrointestinal isolates of *K.pneumoniae* correspond liver isolates genotypically and phenotypically (serotype) as well [22], supporting previous hypotheses that most systemic infections seed from the gut primarily [23]. A widely investigated pathogenicity factor is the capsular (serotype k type) component, which impairs phagocytosis and protects against the bactericidal effects of host

serum [24]. In addition, as was shown in [25], the type VI secretion system helps with colonization throughout the gastrointestinal tract, and the capsule polysaccharide is essential for large intestine colonization.

Conclusion

Albizia lebbeck leaf extract revealed to give an appropriate protection against inflammation as well as damage of virulent *Klebsiella pneumoniae* contagion in comparison to both of KWCSAg-KP and control group.

Disclosure

None

References

1. Cheng F, Li Z, Lan S, Liu W, Li X, Zhou Z, et al. Characterization of Klebsiella pneumoniae associated with cattle infections in southwest China using multi-locus sequence typing (MLST), antibiotic resistance and virulence-associated gene profile analysis. Braz. J. Microbiol. 2018;49:93-100.

2. Paczosa MK, Mecsas J. Klebsiella pneumoniae: going on the offense with a strong defense. Microbiol. Mol. Biol. 2016;80(3):629-61. https://doi.org/10.1128/MMBR.00078-15.

3. Fang C-T, Lai S-Y, Yi W-C, Hsueh P-R, Liu K-L, Chang S-C. Klebsiella pneumoniae genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin. Infect. Dis. 2007;45(3):284-93. . https://doi.org/10.1086/519262

4. Mansour AM, Zaki HM, Hassan NA, Al-Humiany AA. Molecular characterization and immunoprotective activity of capsular polysaccharide of Klebsiella Pneumoniae isolated from farm animals at Taif Governorate. Am. J. Infect. 2014;10(1):1. doi:10.3844/ajidsp.2014.1.14

5. Hayati M, Indrawati A, Mayasari NLPI, Istiyaningsih I, Atikah N. Molecular detection of extended-spectrum β-lactamase-producing Klebsiella pneumoniae isolates of chicken origin from East Java, Vet. World. 2019;12(4):578. https://doi.org/10.14202%2Fvetworld.2019.578-583

6. Sharma S, Kataria A, Shringi B, Nathawat P, Bhati T, Mohammed N. Detection of hypermucoviscous Klebsiella pneumoniae in camel (Camelus dromedarius) during an outbreak of acute respiratory tract infection. JCPR. 2013;20(2):139-43.

7. Ibrahim Z. Experimental infection of Klebsiella pneumoniae in urinary tract of rats and guinea pigs: Ibrahim ZI and Alwaan MJ. Iraqi J. Vet. Med. 2008;32(2):68-79.

8. Pan Y-J, Lin T-L, Chen Y-H, Hsu C-R, Hsieh P-F, Wu M-C, et al. Capsular types of Klebsiella pneumoniae revisited by wzc sequencing. PloS one. 2013;8(12):e80670. https://doi.org/10.1371/journal.pone.0080670

9. Lee C-R, Lee JH, Park KS, Jeon JH, Kim YB, Cha C-J, et al. Antimicrobial resistance of hypervirulent Klebsiella pneumoniae: epidemiology, hypervirulence-associated determinants, and resistance mechanisms. Front. cell. infect. microbiol. 2017;7:483. https://doi.org/10.3389/fcimb.2017.00483

10. Lundberg U, Senn BM, Schüler W, Meinke A, Hanner M. Identification and characterization of antigens as vaccine candidates against Klebsiella pneumoniae. Human Vaccines & Immunotherapeutics. 2013;9(3):497-505. https://doi.org/10.4161/hv.23225

11. Al–Samrraee IAA. Immune response interaction of Klebsiella pneumoniae and Eimeria tenella. Iraqi J. Vet. Med. 2017;41(1):17-22.

12. Jaafar SW, Abdullah AH. Molecular Detection of Pathogenic in Domestic Escherichia coli Pigeon in Baghdad, Iraq Columba livia. Indian Journal of Ecology. 2022. 00 Special Issue (00) 000-000

13. Watban H, Al-Maaly N. Bacteriological identification and molecular detection of Klebsiella pneumoniae from pneumonic camels in Al-Muthanna province. Adv Anim Vet Sci. 2023;11(11):1881-6.

14. Mahdi NR. The Cellular Immunoprotection of BALB/C mice vaccinated with Salt-Extractable Brucella abortus S19 antigens and Immunoadjuvant βeta-glucan challenged with Brucella abortus Virulent Strain: Nidhal Raoof Mahdi and Sabrin Ibraheem Mohsin. Iraqi J. Vet. Med. 2015;39(2):79-86.

15. Al-Samarrae EA. Humoral immune response of Salmonella typhimurium and Salmonella enteritidis sonicated antigens in rabbits: AL-Samarrae. EAA., AL-Shawi, AMA and AL-Taiy, HSR. T . Iraqi J. Vet. Med. 2012;36(0E):84-8.

16. Humad TJ, Hashim MS, Jafar Karim AK. IMMUNO-PATHOLOGICAL EXAMINATION OF TNF ALPHA IN INFECTED AND VACCINATED MICE WITH KELEBSIELLA PNEUMONIAE IN BAGHDAD, IRAQ. Biochem. Cell. 2020;20(1).

17. Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology. Manual of histologic staining methods of the Armed Forces Institute of Pathology1968. p. xii, 258-xii, .

18. 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. Iraqi J. Vet. Med. 2022;46(2):12-9. https://doi.org/10.30539/ijvm.v46i2.1399

19. Hsu H, Nakoneczna I, Guo Y-n. Histopathological evidence for protective immunity induced by sonicated Salmonella vaccine. Can. J. Microbiol. 1985;31(1):54-61. https://doi.org/10.1139/m85-012.

20. Al-Taee K, Al-Dulaimi K. Study the histological changes in intestine of infected rabbits with entamoeba histolytica after immunization with klebsiella pneumonia k-antigen. Adv Anim Vet Sci. 2020;8(11):1250-5. https://doi.org/10.17582/journal.aavs%2F2020%2F8.11.1250.1255

21. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J. Clin. Microbiol. 2007;45(9):2761-4. https://doi.org/10.1128/jcm.01228-07

22. Janda JM. The genus Klebsiella: an ever-expanding panorama of infections, disease-associated syndromes, and problems for clinical microbiologist. Clin Microbiol Case Rep. 2015;1:2.

23. Shon AS, Bajwa RP, Russo TA. Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: a new and dangerous breed. Virulence. 2013;4(2):107-18. https://doi.org/10.4161/viru.22718

24. Yu W-L, Ko W-C, Cheng K-C, Lee C-C, Lai C-C, Chuang Y-C. Comparison of prevalence of virulence factors for Klebsiella pneumoniae liver abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. Diagn Microbiol Infect Dis. 2008;62(1):1-6. https://doi.org/10.1016/j.diagmicrobio.2008.04.007

25. Calderon-Gonzalez R, Lee A, Lopez-Campos G, Hancock SJ, Sa-Pessoa J, Dumigan A, et al. Modelling the gastrointestinal carriage of Klebsiella pneumoniae infections. Mbio. 2023;14(1):e03121-22. https://doi.org/10.1128/mbio.03121-22