Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Comparison between efficiency of propolis extracts and antibiotic treatment of *Klebsiella pneumonia* in rats

Assad Mohan Mohammed Mohammed jawed Al-jobbery
Department of Pathology and Poultry / University of Baghdad - College of Veterinary Medicine / Iraq
Corresponding Author email: asaadmohan2@gmail.com
(Received 14/04/2019, Accepted 19/05/2019)

Abstract

In order to determine the influence of propolis extracts when comparative with antibiotic on Klebsiella pneumonia infection as forty five of white rats, both sexes were randomly divided into three groups of (15) rats each group and were treated as following: 1st groups (n=15) group was inoculated with 1X108 CFU of viable virulent K. pneumoniae, I/P and at 24 hour post-treatment, this group was treated with propolis extraction (100 mg/kg B.W) 1cc / orally /daily, 2nd group (N=15) was inoculated with K. pneumoniae as in 1st group but at day 24 hour post-infection, it was treatment with amoxicillin (100 mg/Kg , B.W), 3ed group(n=15) was inoculated I/P with 1X108 CFU of viable virulent K. pneumoniae and served as control group. Tthe serum levels of TNF a, IL 10 by ELAS Assay in addition to Histopathological examination, result from our study showed elevation TNFa and IL 10 in the first group, Severe pathological lesions were seen in examined organs of control group but these lesions are mild or few in animal treatment with propolis extraction, The main lesions in examined organs of these animals are suppurative inflammation, small granuloma. We conclude that animal treatment with propolis extraction better than antibiotic treatment and control group .

Keywords: Antibiotic, *Klebsiella pneumonia*e, propolis .

Introduction:

Klebsiella pneumoniae is opportunistic Gram negative bacteria belong to Enterobacteriaceae family and it is considered as a major health threat worldwide, (1) this pathogen is the most common etiology of nosocomial respiratory tract infections in addition ,it is the second cause of urinary tract infections and bacteremia Center for Disease Control (2) also is considered a major pathogen that cause high morbidity and mortality (>50%) in the intensive care units,surgical wards and pediatric (3). High morbidity and mortality caused by these strains even in the found sensitive antibiotic strains, these strains possess anti-phagocytosis virulent factors CPS which are mediated resistance complement activity also these pathogen cause severe infections such as pyogenic liver abscess, endophthalmitis and pneumonia (4) Treatment infections by these pathogen are limited due to high incidence of multidrug resistant of *Kelpsiella pneumonia*cross the world (5). Particularly, hypervirulent strains (hvKP) which form a major threat in Asia and Western countries (6) antibiotics, which are the one most important agents using in control pathogen infection, today, become less effective therapy against certain bacteria due to emergence bacterial drug resistant, therefore the researches focus to find new drug therapy lesser toxicity and bacterial resistance ,traditional medicine, particularly herbs sources, is considered the important primary healthcare system in numerous developing countries (7), (8) the natural products of plants showed very successful results against many infectious disease and cancer (9) numerous researchers studied the efficiency

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

of plant extracts in treatment of bacterial infection in the world particularly ethnomedical plants in the India (10). propolis is a name used to mixture of resinous substances that collected from parts of plants by honeybees also it collected from exudates and buds in the north temperature zone, the sources of these substances are willow, beech ,horse chestnut tree, polar, birch and alder (11) a,the hive used the propolis to prevent entrance of intruders such as snakes and Lizards ,,to coat the inner walls, to prvent fungi and bacteria growth and against wind and rain. propolis is used in traditional medicine due to it has a broad spectrum pharmacological activity including antioxidative, antibacterial, anti-inflammatory and antihepatotoxic activity (12) antibacterial activity of propolis is return to its containing of phenolic compounds, especially flavonoids, phenolic acids, and their esters (13) investigated that ani bacterial activity of propolis result from synergistic action between flavonoids and other compounds present in these extract also (14) a reported that propolis expressed inhibitor activity against Candida albicans and Streptococcus mutans with anti-inflammatory effects In Iraq, there is little researchs , in vivo,about the antibacterial activity of propolis,therefore the aim of the present study is to determine the efficiency of propolis in treatment of *K.pneumonia* infection in rats as compared with activity of antibiotic .

Materials and Methods:

Ethical approval

The Animal Ethical Committee has approved the present study.

Bacterial strain :

It is isolated from bovine lung and diagnosis by routine bacteriological methods and confirm diagnosis by biochemical tests and P.C.R which used primer K1 gen primer set (F-5'- GGTGCTCTTTACATCAT and R-5'- GCAATGGCCATTTGCGTTAG) and K2 gen primer set (F-5'- CAACCATGGTGGTCGATTA and R.TGGTAGCCATATCCCTTTGG). this primers used to detection K1 and K2 gene in Kelbsiella pneumonia as (1283 bp product size for K1, 531bp product size for K1).

Bacterial cultivation:

K. pneumoniae was cultivated in NB (in one-liter flask) under shaking condition at 37°C for 72 hrs.

Determine bacterial pathogenicity:

These strain was inoculated several time in mice till the animals were died and the infective dose was prepared from dead animals and challenge dose dose (1X108 cfu/ml) were determine according to (40).

Ethanolic extraction of propolis:

Crude Propolis samples were collected from local beehives of honey bees in Al-Diwanyia city, Iraq during after that, propolis samples were cleaned, free of wax, paint, wood, cut into small pieces. Twenty gram of crude propolis were mixed with 100 ml of ethanol alcohol (70%) in dark brown bottle for extraction. The mixture was left for 7 days at room temperature and in dark place. the container was shaked 2 or 3 times per day and returned to warm dark place. The mixture was filtered through Whatman No. 1 filter paper. This process was repeated twice. Finally the alcohol was evaporated by oven at 45°C, then the extract was weighed and stored in dark clean container until use (41).

Experimental designs:

Fourty five rats, both sexes, were supplied by the animal house of (Babylon University-college of science). Their ages at the start of experiments were 15 weeks, and their weight was 250-300 grams and prior to use. The animals were acclimatized for 7 days at 12 h light/dark cycle. The animals were housed in plastic cages in an air-conditioned room with temperature maintained at 25±2 C. Rats were given food pellets and water ad libitum. All rats were randomized divided into three groups equally and treatmentas following: 1st group was inoculated with 1X10 8 cfu of viable virulent K.pneumoniae, I/P and at 24-hour post treatment, this group was treatment with propolis extraction (100 mg/Kg B.W) orally /daily. 2nd group was inoculated with

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

K.pneumoniae as in 1st group but at day 24-hours post infection, it was treated with amoxicillin (100 mg/ Kg, B.W), orally /daily. 3rd group was inoculated I/P with 1X10 8 cfu of viable virulent K.pneumoniae, and served as control group. At 8 day post inoculation, all animals were sacrificed and blood samples were collected for determine the serum levels of TNF a and IL 10 by, indirect, ELISA Aassay and small pecies were taken from certain organs and fixed in 10 percentage of formalin for Histopathological examination according to (15).

TNFa and IL10 levels:

TNFa and LI10 levels in different treatments were measured according to manufacturers, instructions using Rat TNFa and LI10 platinum ELISA Ready- to -use indirect ELISA, Melsin Medical Co. Limited, chin).

Results:

Cytokine levels:

The result showed that Infected animals treated with propolis extracts expressed high levels of serum TNFa) 3.3±49.9 (than those low levels in the infected animals treated with antibiotic (25.33± 4.58) but the levels of this cytokine in both groups were higher than those in control negative group(2.8 ± 0.37) . The serum levels of IL 10 in infected animal treatment with antibiotic were higher (58.5± 6.06) than those in infected animals treated with propolis extracts (42.25± 2.59) and control group (13.33± 3.17). Table (1).

Table (1):Serum levels of TNF a and IL 10 in treatment infected animals and control negative group at day (8) post infection , fourty five rats, both sexes, were randomized divided into three groups of 15 rats

Groups	IL10	TNF alpha
G1	42.25± 2.59 C	49.9 ± 3.3 B
G2	58.5± 6.06 D	25.33±4.58 C
G3	13.33±3.17 AC	2.8 ± 0.37 C

P≤0.05. The different letters in different groups = significant differences at
LSD = 14.307 LSD =22.323

Histopathological examination :

Animals treatment with propolis extracts:

The lung showed marked hyperplasia of bronchial associated lymphoid Figure(1), in addition to proliferation of alveolar macrophages in alveolar sepaces Figure (2). In othere animals ,the main lesion in the lung were epithelioid granulomatous lesions consisting from aggregation of epithelioid cells in the interstitial tissue Figure (3). Spleen sections howed marked hyperplasia of white pulp Figure (4). The liver revealed small mature granuloma consisting from aggregation active macrophagesytes and lymphocytes in addition to proliferation of kupfer cells Figure (5) and the spleen expressed marked hyperplasia of white pulp Figure (6) Infected animals treatment with antibiotic: Section in lung of animal treatment with antibiotic at 8 days post infection showed thrombus in blood vessels, increase thickness wall of blood vessels due to fibrosis and increase thickness of interalveolar septa due to inflammatory cells infiltration Figure (7) in addition to aggregation of mononuclear cells particularly foamy macrophages in the interstitial tissue Figure (8) as well as neutrophils ,macrophagesand lymphocytes in and around blood vessels Figure (9) there was severe necrotic area in liver parenchyma filled with RBCs and inflammatory cells particularly neutrophils Figure (10). The spleen expressed apoptosis of lymphocytes in white pulp Figure(11) also the kidney revealed atrophy of glomerular tufts with dilated Boman space and acute cellular degeneration of epithelial cells Figure(12).

Control animal post infection.

Histopathological section showed neutrophils aggregation in the wall of bronchiol and blood vessels Figure (13). kidney section showed neutrophils infiltration around glomerula with severe vacuolar degeneration of epithelial cell of renal tubules Figure(14).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

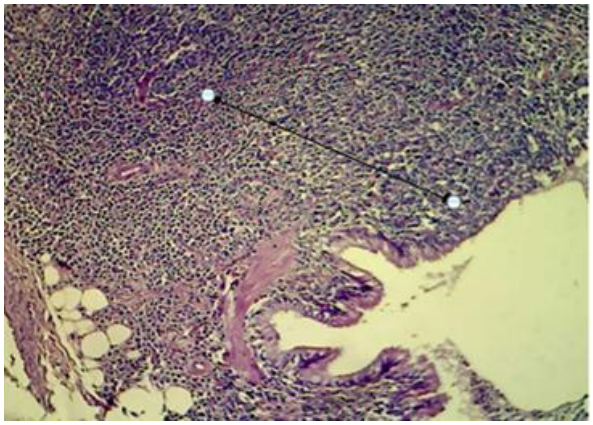


Figure (1): Section in lung of animal treatment with propolis at 8 days post infection shows marked hyperplasia of bronchial associated lymphoid (H & E stain 400X)

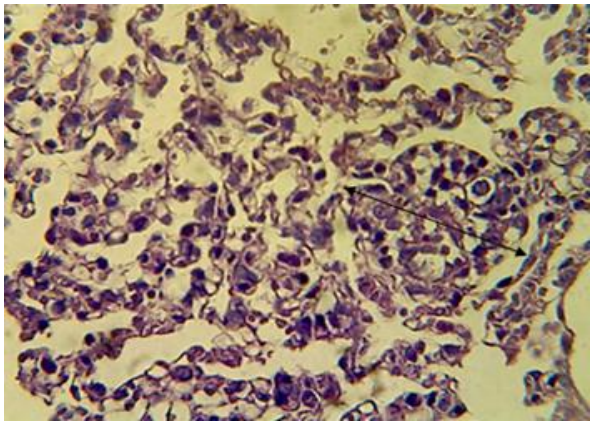


Figure (2): Section in lung of animal treatment with propolis at 8 days post infection shows granulomatous lesion in the interstitial tissue with proliferation of alveolar macrophages in alveolar sepaces (H & E stain 400X)

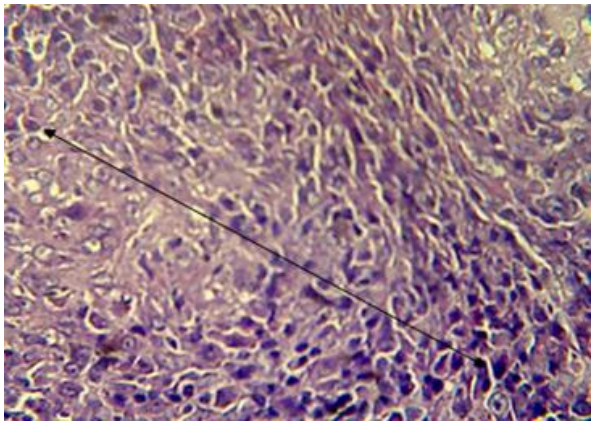


Figure (3): Section in lung of animal treatment with propolis at 8 days post infection shows epithelioid granuloma in the interstitial tissue (H & E stain 400X)

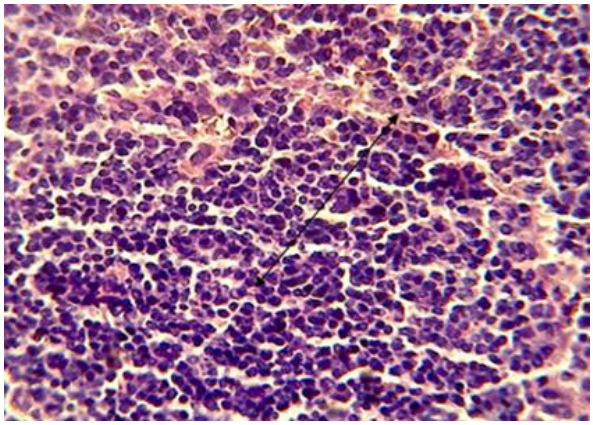


Figure (4): Section in kidney of animal treatment with propolis at 8 days post infection shows aggregation of mononuclear cells around glomerula and between renal tubules (H & E stain 400X)

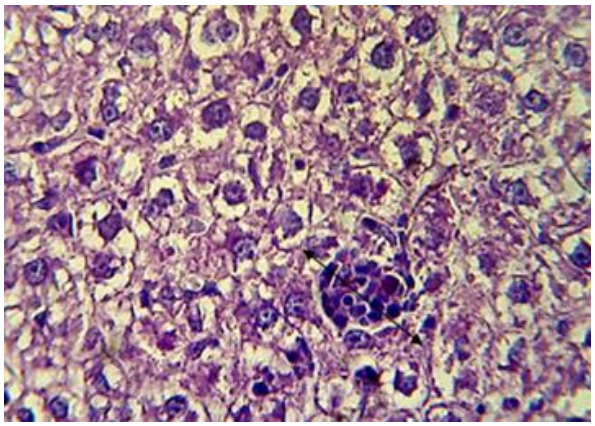


Figure (5):Section in liver of animal treatment with propolis at 8 days post infection shows small mature granuloma consisting from aggregation active macrophagesytes in addition to proliferation of kupfer cells (H & E stain 400X

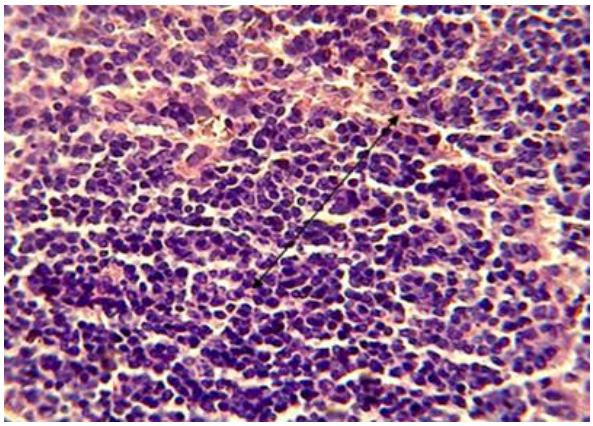


Figure (6): Section in spleen of animal treatment with propolis at 8 days post infection shows marked hyperplasia of white pulp (H & E stain 400X)

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

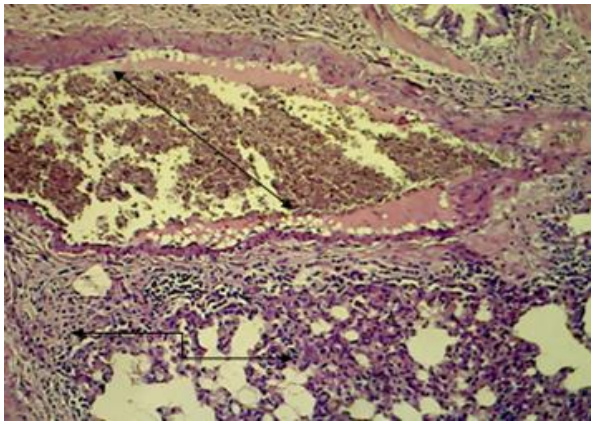


Figure (7): Section in lung of animal treatment with antibiotic at 8 days post infection shows thrombus in blood vessels, increase thickness wall of blood vessels due fibrosis and increase thickness of interalveolar septa due to inflammatory cells infiltration (H & E stain 400X)

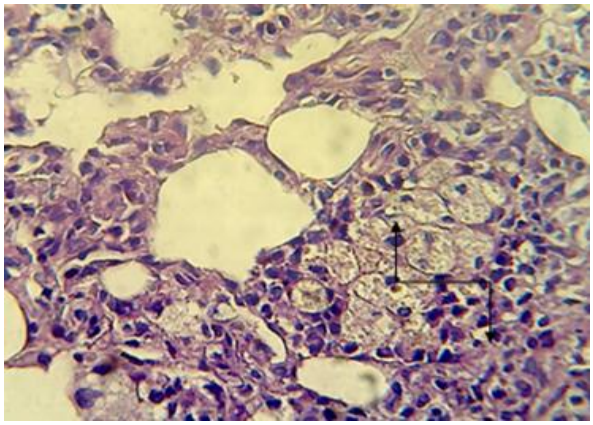


Figure (8): Section in lung of animal treatment with antibiotic at 8 days post infection shows aggregation of mononuclear cells particularly foamy macrophages in the interstitial tissue (H & E stain 400X)

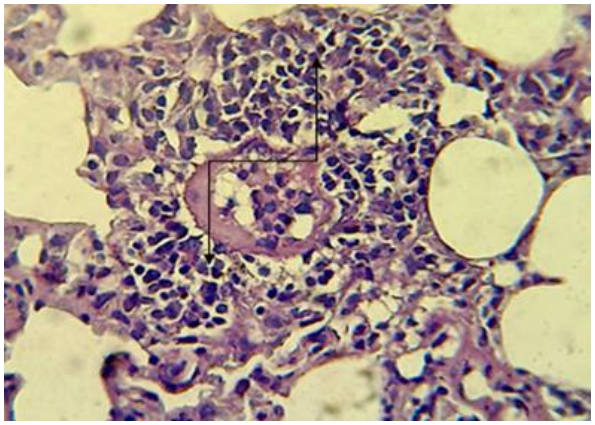
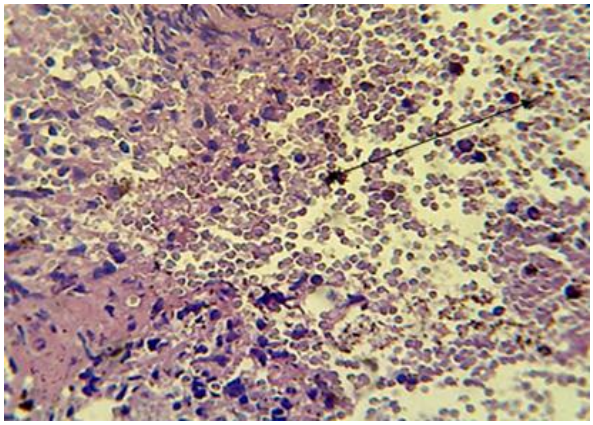


Figure (9): Section in lung of animal treatment with antibiotic at 8 days post infection shows aggregation of neutrophils, macrophagesand lymphocytes in and around blood vessels (H & E stain 400X)



Figure(10): Section in liver of animal treatment with antibiotic at 8 days post infection shows neutrophils and RBCs in large necrotic area (H & E stain 400X)

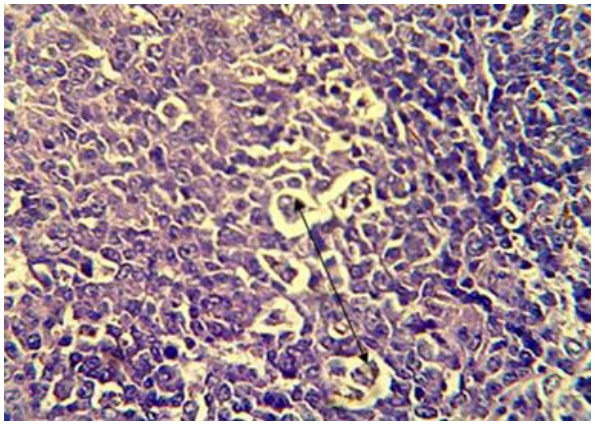


Figure (11): Section in spleen of animal treatment with antibiotic at 8 days post infection shows apoptosis of lymphocytes in white pulp (H & E stain 400X)

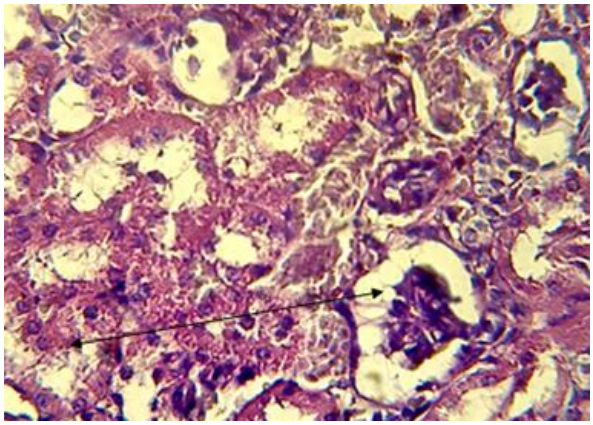


Figure (12): Section in kidney of animal treatment with antibiotic at 8 days post infection shows atrophy of glomerular tufts with dilated Boman space and acute cellular degeneration of epithelial cells (H & E stain 400X) .

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

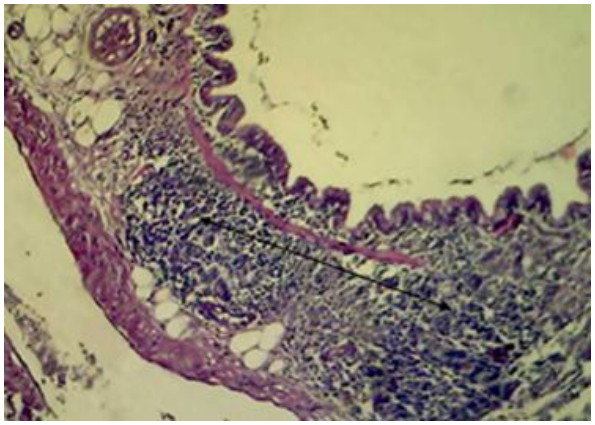


Figure (13):Section in lurg of non immunized animal at 10 days post infection shows neutrophils aggregaton in the wall of bronchiol and blood vessel (H&Estain 400X)

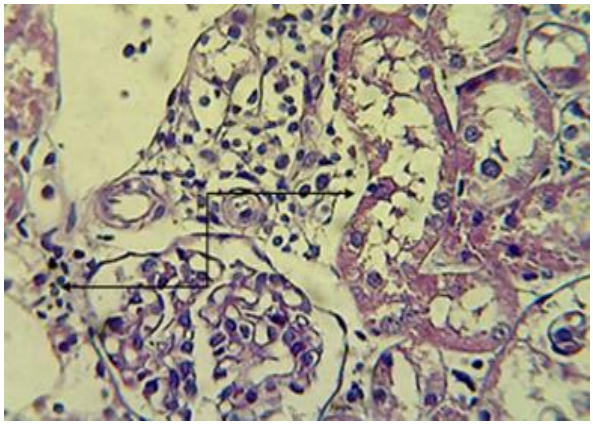


Figure (14): Section n kedney of non immunized animal at 10 days post infection shows neutrophils infltraton around glomerula with severe vacuolar degeneration of epithelial cells of renal tubules (H&E stain 400X)

Discussion:

The present finding expressed that the infected animals treatment with propolis extracts showed high levels of serum TNFα as compared with those levels in infected animals treatment with antibiotic ,but the levels of the IL 10 in these group were higher than TNFα, these result may indicated that the proplis extracts may stimulated immune response and they act as balance between immune response and inflammatory response induced by *Klebsella pneumonia* infection. these idea was agreement with observation of (16) (17) who recorded that the host immune homeostasis can maintained by IL 12 and IL 10 however, the systemic infection induced imbalance immune responses, therefore, it was required to achieve a balance between pro inflammatory and anti -inflammatory cytokines (18) inflammation was considered one type of innate immune response but also it cause tissue damage, there are numerous strategy of prevention inflammatory disease such as probiotic, plant extracts that modulate immune response through activation of phagocytic, NK cells and maturation of DCs. The levels of serum both TNF a and IL 10 in infected animals treated with propolis extracts as compared with those levels in infected animals treated with antibiotics may indicate this substance killed most *K. pneumoniae* and stimulated IL 10 production which is mediated resolution of the infection and it prevents tissue damage, these idea was inconsistent with who demonstrated that anti-inflammatory cytokines and IL 10 can downregulate the proinflammatory cytokines and control tissue damage .moreover, . in pathogenic infection, IL 10 can achieve a balance between Th1 and Th2 response IL 10 inhibit pro-inflammatory cytokines and moderate immunopathology induced by potential Th 1 response against infections also, IL 10 drives antibodies from activation B lymphocytes (19) also the serum IL 10 in infected animals treated with propolis extracts in the current study may give indication that these extracts may activate Th2 cells which play a crucial role in regeneration of tissue injury, these idea was agreement with (20) who found that IL 10 help clonal proliferation and maturation of Th2 cells. That produced IL 4, ILFIV, IL SIX and IL 13, all of these cytokines facilitate antibody production and alternative activated macrophages M2 which secret transforming growth factor beta and other growth factors that responsible for resolution infection and healing process (21,22) also, propolis expressed anti-inflammatory effects according to its geographic region, it

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

activated the production of IL 4 and IL 10 that related to activated Th2 cells (14). The current finding revealed that the levels of serum TNF a in infected animals treatment with antibiotic (25.33±4.58) were lower than those levels in infected animals treatment with proplis extrats (49.9±3.3) these result may indicated that KP infection can lower host innate immune response since TNF a were secreted by phagocytic cells in response to infections, Also the present finding showed high levels of serum 1L 10 (58.5±6.06) in these as compared with those levels in control negative group (13.33±3.17), these result may indicated that these antibiotic drug is insufficient in complete killing KP infection which associated wih stimulated production of IL 10 in order to overcome host defense mechanisms, these evidence was in consistent with(30) who recorded that IL 10 play role in microbial pathogenesis during prevent clearance the pathogen by adaptive immune response, also the present result revealed imbalance between levels of TNFa and IL 10 in these group, also intimate cross regulation of homeostasis and pathology can perform by pro inflammatory cytokines such as TNF a and anti-inflammatory cytokines such as IL 10 that produced by TH1 and Th2 responses respectively (23). the imbalance between Th1 and TH2 responses associated survive and persistent infection with chronic inflammation (24). The current study may be indicated that *Kelpsiella Pneumonia* is considered one pathogen can stimulated differentiated macrophages into alternative activated type that secreted IL 10, these idea was dependent on observation of (23) who demonstrated that IL-10 is derived from a number of cellular sources relating to the type of infection, the type of host cell that the microorganism or foreign epitope comes into contact with them, and the signal transduction pathway (s) initiated , also they showed that certain pathogen can stimulated the phagocytic cells to secrete selected cytokines that favors their survival in the host tissues such as IL 10, also it was reported that cell activation and differentiation can influence by IL 10 secretion by APCs (25). The current finding revealed that lymphoid tissue hyperplasia in the lung, kidney and spleen of infected animals treatment with propolis extracts, these result may be indicated that these extracts can st immune response, these idea was agreement with (26) who demonstrated that the propolis possess immunomodulatory and immunostimulatory activity and increases the ratio of CD4/CD8 T cells in mice, in addition, granulomatous lesions present in the lung and liver in the present finding may also be indicated that propolis extracts stimulated the immune response that limited the infection through granulomatous reaction which associated with cellular immune response practically these group expressed high levels of TNFa that play important role in granulomatous formation .K. pneumonia was considered extra cellular and intracellular pathogen, cell mediated immune response play role in eradicated intracellular pathogens. Cell mediated immunity (Th1 response) associated with activity of phagocytic cells to kill the invasion pathogen through release of of toxic free radicals including reactive oxygen species and reactive nitrogen species RNS) (21) the above evidences were agreement with [27]who recorded that NFa act synergy with INF y to increase microbicidal activity of phagocytic cells and stimulate production of reactive nitrogen intermediates by macrophages also TNF a play important role in extravasation process and migration of immune cells to the site of injury and induce granuloma formation (28). However, it was recorded that mild lesions appear in the animals treatment with Propolis extracts , these result may indicated that these extracts may killed *K. pneumoniae* through activated macrophages, these evidence was agreement with (29) who

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

demonstrated that the immunostimulatent activity of the propolis related to activate the macrophages that lead to increase their phagocytic capacity also propolis can activated cells to produce high levels of H2O2(30). Also the pathological lesions in the infected animals treatment by propolis extracts in the present study may indicated the extracts have antimicrobial activity, these idea was in consistent with observation of (31) who demonstrated that propolis extracts were able to inhibit biofilm formation by *Pseudomonas aeruginosa* and expressed cytoxic effects on these pathogen, the propolis expressed numerous therapeutic features including anesthetic, anticariogenic antibacterial, anti-inflammatory, healing, antifungal, antiprotozoan and antiviral activities antibacterial activity against both Gram negat Gram positive bacteria related to synerm between its compounds such as pinocembrin and galangin flavonoids in addition antl activity result from activity of chrysin and kaempferol that cause reduction of intracellular proliferation of some viruses, such as herpes simplex (32) and it is consider potential anti-inflammatory agents in acute and chronic stage of inflammation (33) ،The mild pathological lesions in the examined organs of infected animals treatment by propolis in the current result may due due these extracts has ant oxidative activity ,these idea was agreement with who recorded that propolis can inhibit ROS production due to higher contain of chrysin and galangin also (33) investigated that the propolis and bud poplar resins extracts can used as adjuvant in the treatment *P. aeruginosa* chronic infection due to its antibi film activity, and its biological properties as anti-inflammatory and antioxidant properties and its low toxicity also (34) (recorded that 1% honey concentration can activated monocytes to secreted pro inflammatory cytokines including TNFa, IL six and IL 1 that activated immune responses Inflammatory reaction also associated with releasing [35]free radical during infection that lead to oxidative tissue damage, flavonoid like compound is a major compound of propolis that act as antioxidant and anti-inflammatory agent (36). The necrotic hemorrhagic hepatitis in addition to thrombus formation, apoptosis and supportive reaction in the animals treatment ampicillin post infection in the present finding may indicated these of *K.pneumoniae* strain characterized by marked antibiotic resistance features that do not killed by these antibiotic. these idea was agreement with the observation of (38) who recorded that *Klebsiella pneumonia* characterized by highly antimicrobial drug resistance particularly against cephalosporins and fluoroquinolones, therefore alternative methods are required including herbal extracts to combat bactral resistant (39), the current study revealed the propolis extracts can protective the animals against K.pneumonia infection better than an antibiotic, these result was similar to the finding of (37) who invested that antibiotics drugs can succeed in the treatment the bacteria in the acute phase of infection (planktonic cells life form) but the bacteria become resistant to these drugs in the sessile aggregate form (biofilm) as in chronic infection , and they suggested other traditional treatment systems were required to controlling bacterial infections , propolis can inhibited bacterial biofilm formation such as S. mutants, by inhibit their virulence factors including lipase and coagulase (1) the present study concluded that propolis extract expressed high antimicrobial activity against *Kelpsiella pneumonia* infection associated balance regulated bet ween serum levels of INF a and IL 1.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

References

1-Scazzocchio F, et al., Multifactorial aspects of antimicrobial activity of propolis. *Microbiological research*, 2006; 161(4): p. 327-333.

2-Huang Y, et al., Rapid detection of K1 hypervirulent Klebsiella pneumoniae by MALDI-TOF MS. *Frontiers in microbiology*, 2015; 6: p. 1435

3-Palusiak A. The antigens contributing to the serological cross-reactions of Proteus antisera with Klebsiella representatives. *Molecular immunology*, 2015; 64(1): p. 228-234.

4-Wang L. Resistance of hypervirulent Klebsiella pneumoniae to both intracellular and extracellular killing of neutrophils. *PloS one*, 2017; 12(3): p. e0173638.

5-Vieira AT. Control of *Klebsiella pneumoniae* pulmonary infection and immunomodulation by oral treatment with the commensal probiotic Bifidobacterium longum 51A. *Microbes and infection*, 2016; 18(3): p. 180-189.

6-Diago-Navarro E. Antibody-based immunotherapy to treat and prevent infection with hypervirulent *Klebsiella pneumoniae*. *Clin. Vaccine Immunol.*, 2017; 24(1): p. e00456-16.

7-Houghton PJ. The role of plants in traditional medicine and current therapy. *The Journal of Alternative and Complementary Medicine*, 1995; 1(2): p. 131-143

8-Dubey N, R Kumar, P Tripathi. Global promotion of herbal medicine: India's opportunity. *Current science*, 2004; 86(1): p. 37-41

9-Runyoro DK. Screening of Tanzanian medicinal plants for anti-Candida activity. *BMC complementary and alternative medicine*, 2006; 6(1): p. 11.

10-Zhang M. Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. *Biomaterials*, 2016; 101: p. 321-340.

11-Ahmad TA. Development of immunization trials against Klebsiella pneumoniae. *Vaccine*, 2012; 30(14): p. 2411-2420.

12-Bankova V. Recent trends and important developments in propolis research. *Evidence-based complementary and alternative medicine*, 2005; 2(1): p. 29-32.

13-Velikova M. Chemical composition and biological activity of propolis from Brazilian meliponinae. *Zeitschrift für Naturforschung C*, 2000; 55(9-10): p. 785-789.

14-Liberio SA. Antimicrobial activity against oral pathogens and immunomodulatory effects and toxicity of geopropolis produced by the stingless bee Melipona fasciculata Smith. *BMC Complementary and Alternative Medicine*, 2011; 11(1): p. 108.

15-Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 1968.

16-Sparo M. Characteristics of an environmental strain, Enterococcus faecalis CECT7121, and its effects as additive on craft dry-fermented sausages. *Food microbiology*, 2008; 25(4): p. 607-615.

17-Castroa M. Enterococcus faecalis CECT7121 induces systemic immunomodulatory effects and protects from Salmonella infection. *International Journal of Probiotics and Prebiotics*, 2007; 2(4): p. 215.

18-Barton GM. A calculated response: control of inflammation by the innate immune system. *The Journal of clinical investigation*, 2008; 118(2): p. 413-420.

19-Fiorentino DF. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. The Journal of Immunology, 1991; 146(10): p. 3444-3451.

20-Osswald T, S García-Rodríguez, Handbook of Applied Biopolymer Technology ed SK Sharma and A Mudhoo. 2011, Cambridge: *Royal Society of Chemistry*

21-Couper KN, DG Blount, EM Riley. IL-10: the master regulator of immunity to infection. *The Journal of Immunology*, 2008; 180(9): p. 5771-5777.

22-Stijlemans B. A glycosylphosphatidylinositol-based treatment alleviates trypanosomiasis-associated immunopathology. *The Journal of Immunology*, 2007; 179(6): p. 4003-4014.

23-O'garra A, P Vieira. Regulatory T cells and mechanisms of immune system control. *Nature medicine*, 2004; 10(8): p. 801.

24-Fleming SD. Surface interleukin-10 inhibits listericidal activity by primary macrophages. *Journal of leukocyte biology*, 1999; 66(6): p. 961-967.

25-Edwards JP. Biochemical and functional characterization of three activated macrophage populations. *Journal of leukocyte biology*, 2006; 80(6): p. 1298-1307.

26-Toreti VC, et al., Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence-based complementary and alternative medicine*, 2013.

27-Moore TA. γδ-T cells are critical for survival and early proinflammatory cytokine gene expression during murine Klebsiella pneumonia. *The Journal of Immunology*, 2000; 165(5): p. 2643-2650.

28-Ramphal R, et al., Control of Pseudomonas aeruginosa in the lung requires the recognition of either lipopolysaccharide or flagellin. *The Journal of Immunology*, 2008; 181(1): p. 586-592.

29-Scheller S. The ability of ethanolic extract of propolis (EEP) to protect mice against gamma

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

irradiation. *Zeitschrift für Naturforschung C*, 1989; 44(11-12): p. 1049-1052.

30-Ouyang W. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity*, 2000; 12(1): p. 27-37.

31-De Marco S. Antibiofilm and antioxidant activity of propolis and bud poplar resins versus Pseudomonas aeruginosa. *Evidence-Based Complementary and Alternative Medicine*, 2017.

32-Marcucci M, Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie*, 1995; 26(2): p. 83-99.

33-Borrelli F. Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia*, 2002; 73: p. S53-S63.

34-Tonks AJ. Honey stimulates inflammatory cytokine production from monocytes. *Cytokine*, 2003; 21(5): p. 242-247.

35-Angus DC, et al., E5 murine monoclonal antiendotoxin antibody in gram-negative sepsis: a randomized controlled trial. *Jama*, 2000; 283(13): p. 1723-1730.

36-Celik S. Caffeic acid phenethyl ester suppresses oxidative stress in Escherichia coli-induced pyelonephritis in rats. *Molecular and cellular biochemistry*, 2007; 297(1-2): p. 131-138

37-Bjarnsholt T, The Role of Bacterial Biofilms in Chronic Infections (Disputats) .2013; Wiley-Blackwell .

38-Pleszczyńska M, Wiater A, Bachanek T, Szczodrak J. Enzymes in therapy of biofilm-related oral diseases. *Biotechnology and Applied Biochemistry*, 2017; 64(3), 337-346.

39-Karygianni L, Al-Ahmad A, Argyropoulou A, Hellwig E, Anderson AC, Skaltsounis AL. Natural antimicrobials and oral microorganisms: a systematic review on herbal interventions for the eradication of multispecies oral biofilms. *Frontiers in Microbiology*, 2016; 6, 1529.

40-Menisy M, Hussein Ghazy, A Sheweita SA. Amro Abd Al Fattah Amara, Klebsiella pneumoniae Ghosts as Vaccine Using Sponge Like Reduced Protocol. *iMedPub Journals* , 2017; Vol. 3 No. 2: 11.

41-Krell R. Value-added products from beekeepng (No.124). Food & Agriculture Org. 1996.