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Study of the Effect of *Klebsiella pneumoniae* Antigens on the Histological Structure of the Kidney in Laboratory Rabbits Infected with *Entamoeba histolytica*

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Key words: *Entamoeba histolytica*, kidney, *Klebsiella pneumoniae*, antigen.
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ABSTRACT

This study was conducted to investigate the immunological effect of Capsular (K-Antigen), extracted from *Klebsiella pneumoniae*, on the histological structure of the kidney in the adult white rabbits that were experimentally infected with *Entamoeba histolytica*. Twenty Male adult white rabbits, were divided into four groups, five animals for each group. The control group was injected with a physiological saline solution, the second group was injected with *E.hisotlytica*, the third group was injected with K-Antigen extract, the fourth group was injected with K-Antigen extract, *E.histolytica* parasites. The results of the histological examination showed many differences in the histological sections examined for the treated groups.

دراسة تأثير مستضدات بكتريا *Klebsiella pneumoniae* على التركيب النسيجي للكلية في الارانب المختبرية المصابة بطفيلي الاميبا الحالة للنسيج *Entamoeba histolytica*

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الخلاصة

اجريت هذه الدراسة لمعرفة التأثير التمنيعي لمتعدد السكريات المحفظي Capsular (K -Antigen) المستخلص من بكتريا *Klebsiella Pneumoniae* على التركيب النسيجي للكلية في الارانب البيض البالغة التي اصيبت تجريبيا بطفيلي *Entamoeba histolytica* استخدم في هذه التجربة (20) من ذكور الارانب البيض البالغة و التي قسمت الى (4) مجموعات ، (5) حيوانات لكل مجموعة . جرعت مجموعة السيطرة بمحلول الملحي الفسيولوجي ، وجرعت المجموعة الثانية بطفيلي *E.histolytica* ، اما المجموعة الثالثة فحقنت بمستخلص (K-Antigen) ، وحقنت المجموعة الرابعة بمستخلص (K-Antigen) ثم جرعت بطفيلي *E.histolytica* . وقد اظهرت نتائج الفحص النسجي العديد من الاختلافات في المقاطع النسجية المفحوصة للمجاميع المعاملة .

الكلمات المفتاحية : الكلية ، *Entamoeba histolytica*
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Introduction:

The kidney is an excretory organ in humans and invertebrates in general. The kidney had a convex external surface and a roofed inner surface known as the hilum. Each kidney is connected to two blood vessels, one of which is a branch of the anterior artery known as the renal artery, which enters the kidney and branches within it. The other is the renal vein. The kidney works on the disposal of waste products from various metabolic processes in the body, as well as the filtering out foreign chemicals, drugs and waste produced from hormone metabolism(Abu-Zaiton,2010)

The kidney is affected by pathogens such as viruses and parasites, among these parasites *Entamoeba histolytica* which is a single-cell parasite that affects the large intestine, causing diarrhea of varying degrees of severity accompanied by mucus and blood, and causes cases of dehydration, malnutrition, abdominal pain, and can infect other organs via blood circulation, such as the liver,

lungs and heart, causing several changes pathological, physiological, and histological affects in those organs.(Al-Nafouli, 2004)

Inclusion of amoebic disease is very rare since kidneys are the fifth most common site of amoebic abscess (Roitt *etal.*,2001). Few cases of amoebic abscess were reported in the kidneys (Brandt and Tamayo, 1970). It was recorded according to (Sezgin *etal.*, 2003) and (Anuradha *etal.*, 2005) cases of the kidney of the abscess associated with bacterial infection, such as the infection of *K.Pneumoniae* and some diseases, including high blood sugar fluctuating

Different types of immunosuppressants have been used to protect the body from various infections. Among these immunosuppressive agents (K-Antigen), which is caused by *K. pneumoniae*, is a member of the intestinal family Enterobacteriacea for gram-negative bacteria, bronchial, non sporic, containing a capsule of multiple sugars, causing several diseases including pneumonia, urinary tract infection and others (Stoesser *etal.*, 2016). Therefore, the study was designed to find the immunological effect of the bacterial extract which resists the harmful effect of *E. histolytica* on the kidney tissue.

Materials and Methods:

1. Obtaining Bacteria Samples:

Pure bacterial isolation of *K. pneumoniae* was obtained from the Microbiology Laboratory at the Faculty of Applied Sciences / University of Samarra. The diagnosis was confirmed using Api-20E after the development of bacteria on the culture of the heart and brain infusion. Of the presence of the capsule according to method (Atlas, 1995), and was extracted capsule according to the method followed by (Taylor and Juni, 1961) with some modifications made by (Al-Khafaji, 2006), Molisch test was conducted to detect sugars According to his method (Plummer , 1978), and the amount of sugar purified from the capsule extract using the method of phenol-sulfuric acid mentioned by (Dubois *etal.*, 1956).

2. Obtaining Parasite Samples:

E.histolytica parasite samples were obtained from the outpatients and residents of Samarra General Hospital, who suffer from moderate to severe diarrhea and in most cases, have hemodialysis. The samples were identified by the method used by (Singh *etal*., 2009), and then the parasite was isolated according to the method of (Clark and Diamond, 2002). The number of cysts was then calculated and the injection dose was determined. The dose was determined by calculating the number of cysts in the quantity of (0.1) ml and by $4*10^{\wedge 3}$ cyst / dose for each animal that was injected orally. The parasite cysts were examined in infected rabbits for two weeks after the infection to confirm the occurrence of parasitic infection. The infection was confirmed by using the method of flotation with the sugar solution, and then the preparation of several smears on a glass slide and examined under the microscope after dyeing with the solution of Lugol's Iodine to detect the parasite in its various stages.

3. Laboratory Animals:

In this study, males of New Zealand adult white rabbits were obtained from the National Center for Drug Control and Research. The weights of the used animals ranged from 1000 to 1800g and ranged in age from 10 to 18 months. The animals were fed the ready-made formula for the laboratory animals and supplied with food and water continuously throughout the study period.

4. Experiment Design:

Twenty white adult male rabbits were used after confirming their health and that they do not have any apparent diseases. The animals were divided into 4 groups, each group of (5) animals as follows:-

G1:- The control group which was given a physiological saline solution for the duration of the experiment and at a dose of 2 ml daily for two weeks.

G2:- Which was injected orally with the parasitic cysts *E.histolytica* of (0.1) ml and by $4*10^{\wedge 3}$ cyst / dose for each animal. Animal faeces were examined daily to confirm the infection. Two weeks after the injection they were dissected.

G3:- Which was given subcutaneous and intramuscular injections of Antigen-K by (1) ml per animal. The animals were dissected at the end of the immunization period after identifying the toxic concentration of the antigen.

G4:- Which was injected with K-Antigen extract with the same dose given to G3 and then injected with *E.histolytica* with the same dose given to G2.

5 – Obtaining Histological Sections:-

After the experiment, the animals were dissected and samples of the kidney 0.5 cm^3 were taken and placed in formalin 10% for a period of 12 hours, and then washed with water for a period of 10 minutes, followed by a series of passes with alcohol, then xylene and then impregnated with paraffin waxing to made special L-Shaped molds using paraffin wax with a melting point of 60°C, then cut by a microtome with a thickness of 4-5 microns, the sections were fixed with alcohol concentration of 30% and then transferred to a special water bath for brushes of sections at 45°C, And then loaded on glass slides, to be ready for staining with hematoxylin and Eosin by (Bancroft and Stevens,1982). The slides were examined under a light microscope.

Result & Discussion:

Group Control (G1):-

The structure of the control group was characterized by a normal appearance of the cortex and medulla. The cortex was characterized by the presence of the renal glomeruli as well as the natural appearance of the kidneys. The medulla region was also characterized by a natural appearance. **(Fig.1)**

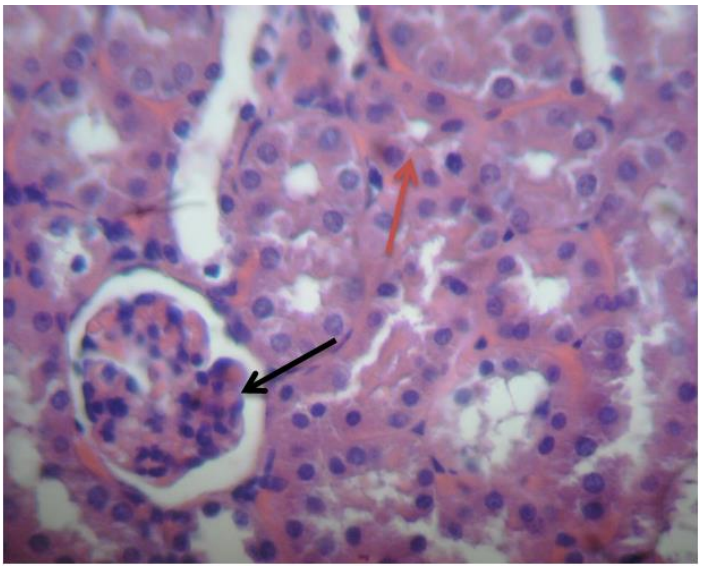

Fig. (1): Section in the control group rabbit's kidney, showing the glomerular (⟶) and kidney tubules (⟶) stained with Hematoxylin - Eosin, (400X).

G2:- The histological sections of the *E.histolytica* group showed degenerative tissue changes with the presence of desquamation in the renal tubules and cell necrosis which appeared in various stages of karyolysis and congestion within nearby renal tubules, whereas the glomerulus appeared close to normal in appearance. **(Fig.2)**

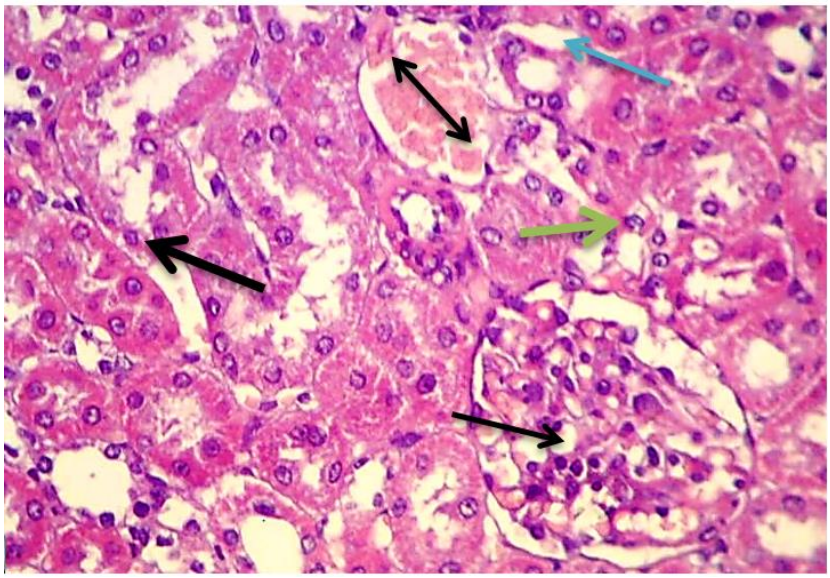

Fig. (2): The histological section in the rabbit kidney, Infected with *E.histolytica*, showing the glomerular ( ), Blood congestion ( ),necrosis ( ), desquamation ( ) , karyolysis ( ). Stained with Hematoxylin - Eosin (400X).

These results were in agreement with that from (Sezgin *etal.*, 2003). The detailed examination of a sample of a kidney taken from a woman infected with parasites revealed that there were many yellow-green abscesses. The histological examination showed signs of necrosis. The study also showed that the glomeruli and vessel structures were close to normal appearance. He also noted that 15 samples taken from a female college suffering from hypercalcemia contained motile trophozoites of *E.histolytica* with characteristic pseudopodia and ingested RBCs were seen in saline wet mount. The background was filled with pyknotic bodies. On cytological examination, degenerated neutrophils and macrophages were seen.

The parasite acted to cause damage in epithelial cells, which were damaged by one of the two mechanisms, either cell death as a direct result of parasite invasion and reproduction or cell damage due to inhibition of the work of renal cells and adjacent inflammatory cells at the site of infection and die from Apoptosis. The products of inflammatory reactions lead to Increased kidney tissue breakdown and thus the occurrence of purulent inflammation (Goodgame, 1996) . The reason for the reduced ability of phytoplasmic cells to prey on the parasites may be due to the increased concentrations of toxic substances and enzymes produced from the same phytoplankton that are likely to have a negative effect on these cells (Lock *etal.*, 1990).

G3:- The histological sections of the group treated with (K-Antigen) showed the appearance of a glomerular renal close to normal in the control group, and also having normal of renal tubules, except the karyolysis and necrosis in some cells (**Fig.3).**

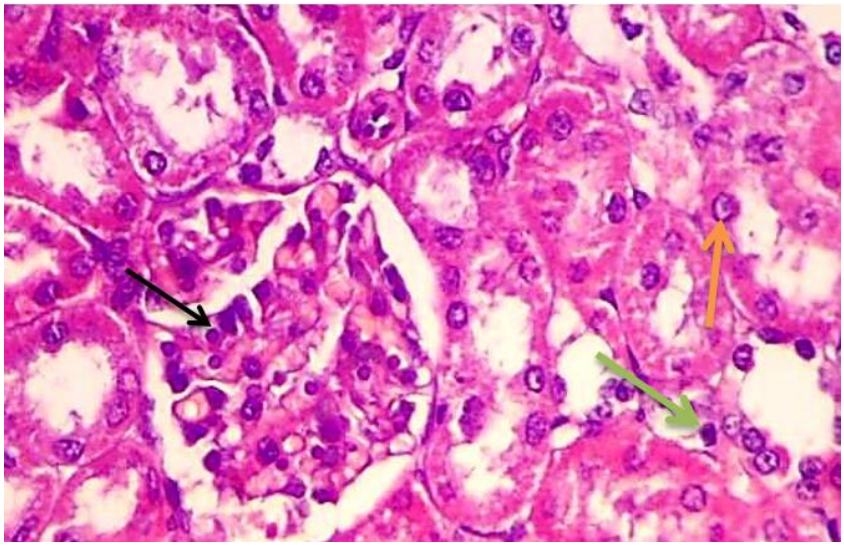

Fig. (3): The histological section in the rabbit kidney, treated with (K-Antigen), showing the glomerular (⟶), karyolysis (⟶), necrosis (⟶). Stained with Hematoxylin - Eosin, (400X).

These results were consistent with those recorded (Al-Kabi , 2006). The antigen of the external membrane proteins and the capsule of K. *pneumoniae* were used as an immunizer in rabbit and observed an increase in the thickness of the foot pad after 48 hours and also observed that immunization of the outer membrane proteins of Brucella ovis 24 - 48 hours more than internal membrane proteins and cytoplasm of these bacteria, and this can be attributed to the effect of sugar on the cells of the pharynx and activation, which in turn increased the number of lymphocytes, which leads to coordination between them and regulate the size of cellular immune response and increases the secretion of a compound (K-Antigen), which can lead to inflammation and the degradation of some cell nuclei. This stimulates the immune system of rabbits positively, as a result of the activity of the cells and the lymphocytes associated with the release of Antrolinks and especially IL10, which confirms the role of antigen k in improving the immune system and that the vaccine has raised the immune status and is non-toxic and has no negative impact on the kidney tissue (Moonah *etal.*, 2013)

G4: The histological sections of the kidneys of animals injected with *E.histolytica* and treated with (K-Antigen) were shown the glomerular renal with a normal appearance in the cortex and medulla regions، as well as the appearance of nearby and distant tubules being normal too **(Fig.4, 5).**

This may be due to the ability of the antigen to cause a reaction or immune response within the kidney tissue that has greatly contributed to the resistance of the damage that could have been caused by the parasite. These results are consistent with those recorded by (Al-Kabi , 2006), showing the ability of polycystic polysaccharides for *K. pneumoniae* on increased phagocytic efficacy, They also agreed with what was previously recorded by (AL-Taei, 1996) where he observed a high rate of phagocytosis in rats immunizing with polysaccharides extracted from *Rhizobium leguminosarum* bacteria before and after infection with hydatid cysts.

The macrophages play a large role in the host's response to Entamoeba, It is considered a pesticide for Entamoeba by stimulating the TNF-α and IFN-γ (Moonah *etal.*, 2013), the antigen acts to stimulate the process of bovine by stimulating the macrophages to produce a large amount of nitric oxide (NO), which is toxic and deadly to Entamoeba. The importance of nitrite oxide produced from macrophages is attributed to its ability to activate the enzyme Ribonucleotide reductase, where it was found to be sensitive to levels Low Nitric Oxide (Serbina *etal.*, 2003).

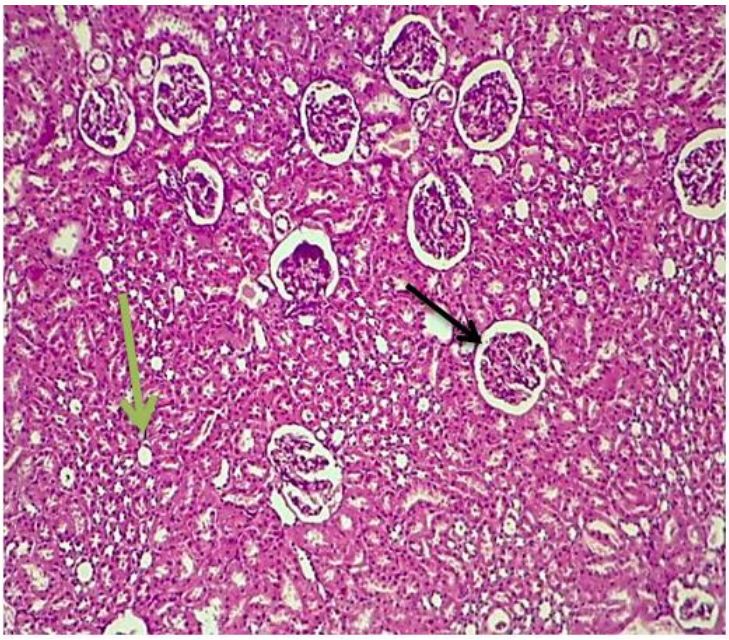

Fig. (4): The histological section in the rabbit kidney from animals injected with *E.histolytica* and treated with (K-Antigen) showing the glomerular renal with normal appearance (⟶) and normal nearby and distant tubules (⟶). stained with Hematoxylin - Eosin, (200X).

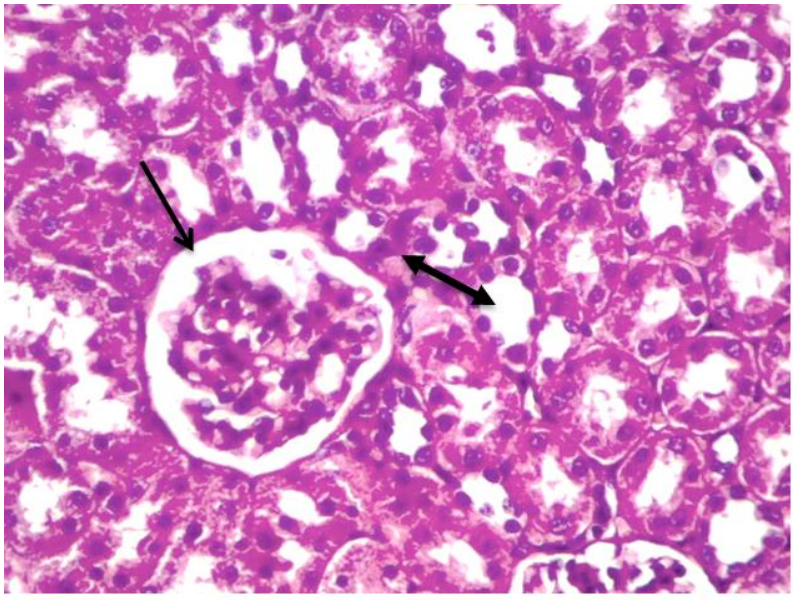

Fig. (5): The histological section in the rabbit kidney from animals injected with *E.histolytica* and treated with (K-Antigen) showing the glomerular renal with normal appearance (⟶) and normal nearby and distant tubules (⟷). stained with Hematoxylin - Eosin, (400X).

References:

Abu-Zaiton, A. S. (2010). Anti-diabetic activity of Ferula assafoetida extract in normal and alloxan-induced diabetic rats. Pakistan Journal of Biological Sciences, 13(2): 97.

Al-Kabi , S. J. M.(2006). A study Of The Effect Of Some Antigens Of *Klebsiella pneumoniae* On The Immune Response. Ph.D. Thesis. Al- Mustansiriya University.(in Arabic).

Al-Khafaji, S.M.S. (2006). Study the capsule bacteria of *Acinetobacter baumannii* and its effect on immune response. Ph.D.thesis, Faculty of Science, Al- Mustansiriya University.(in Arabic).

Al-Nafouli, D.M.Y. (2004). Techniques of different dyes to detect the of *Entamoeba histolytica* tissue with trace histological changes caused by experimental amoebae in mice. Master Thesis, University of Mosul.(in Arabic)

AL-Taei, A. F. M. (1996). Activation of macrophages with immunomodulators and the effect of this activation upon the infection with *Echinococcus granulosus*. Ph. D. Thesis, Univ. AL-Mustansiriya. Coil. Sci. (in Arabic).

Anuradha S. ; Chandel U.K ; Gupta, M.L. ; Vijay S. and Sharma, R. K. (2005). Amoebic Renal Cyst: A Case Report . The Braz. J. Infec. Dis. 9(3):266-268.

Atlas, R.M. (1995). Principles of Microbiology. 1st ed. Mosby-Year Book, USA.

Bancroft, J. and Stevens, A. (1982). The theory and practice of histological techniques. 2nd ed. Churchill Livingstone.p:49-113.

Brandt, H. and Tamayo, R.P.(1970). Pathology of human amebiasis. Hum. Pathol.1 (3): 351–85.

Clark, C . G and Diamond, L. S. (2002). Methods of Cultivation of huminal parasitic protists of Clinical importance. Clin . Microbiol . Rev . , 15 (2) : 329 - 341 .

Dubois , M . ; Gilles , K . L . ; Hamilton , H . K . ; Roberts , P . A . ; and Sman , F . (1956) Colorimetric method for determination of sugars and related substance. Anal. Chem. 28: 350 - 356.

Goodgame, R. W. (1996). Understanding intestinal spore-forming protozoa: cryptosporidia, microsporidia, isospora, and cyclospora. Ann.Int.Med., 124(4), 429-441.

Lock ,R.; Dahlgren ,C.; Linden ,M. ;Stendahl ,O. ;Svensbergh , A. and Ohman, L.(1990) . Neutrophil killing of two type 1 Fimbrio-Bearing *Escherichia coli* strain dependence on respiratory burst activation .Infec.and Immun. ,58 :37-42.

Moonah ,S.N.; Jiang, N.M. and Petri ,W.A.(2013). Host immune response to intestinal amebiasis. PLoS Pathog. 9:e1003489. 10.1371/ Journal. ppat.1003489.

Plummer , D . T . (1978) . An introduction to practical biochemistry . 2nd ed . McGrrow . Hill book Company . U . K England.

Roitt, I.; Brostoff. J.; and Male, D.(2001). Immunology. 6th ed . Harcourt publisher limited. Mosby. London.

Serbina , N.V.; Salazar-Mather , T.P. ; Biron , C.A. ; Kuziel , W.A. and Pamer,E.G.(2003). Producing dendritic cells mediated innate immune defenceTNF/iNOs- against bacterial infection. Immun. 19 :59-70

Sezgin, g.; Ferhat, k.; Fazilet, k.; Ilhan, t. And Hakan, O. (2003). Emphysematous pyelonephritis and renal amoebiasis in a patient with diabetes mellitus. Int. J. Urology. 10: 404–406

Singh, A .; Ericttouft, B.H. and William, A.C. (2009). Rapid diagnosis of intestinal parasitic protozoa .J. Infect. Dis.,61(3): 280-286.

Stoesser, N., Mathers, A. J., Moore, C. E., Day, N. P. and Crook, D. W. (2016). Colistin resistance gene mcr-1 and pHNSHP45 plasmid in human isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *The Lancet infectious diseases*, *16*(3), 285-286.

Taylor , W . H ; and Juni , E . (1961). Pathwaya for biosynthesis of a bacterial capsular polysaccharide . J . Bacteriol . 81 : 688 - 693 .