# STUDY OF SOME PATHOLOGICAL CHANGES IN MICE GROUPS INDUCED BY *MYCOBACTERIUM TUBERCULOSIS* AND TREATED WITH ETHAMBUTOL

Suhair H. Al-kutbi

Department of Clinical Laboratory Science, Collage of Pharmacy ,University of Baghdad, Baghdad,Iraq

(Received 26 June 2010 , Accepted 5 October 2010 )

**Keywords;** histopathological, mycobacterium tuberculosis, granulomatous

## ABSTRACT

The main objective of this study is to demonstrate the histopathological changes and the efficacy of ethambutol of treatment mice infected with mycobacterium tuberculosis.

Thirty of white Swisrland mice is 6-8 weeks age, weighted 20-25gm were used they were randomly divided into 3 groups contain of 10 animals for each group.

- The 1st group (infected animal group) were inoculate.ed with 0.1 ml of bacterial suspension contain $1x10^8$ cfu/ml intraperitonialy.
- The 2nd group (group of infected-treated animal) were also infected as the fist group but after 30 day of infection were treated with 0.1 ml of ethambutol was given orally for 1 30 days.
- The 3rd group maintained as a control and were inoculated with 1 ml of sterial normal saline intraperitonealy.

At 60 day post infection all animals were sacrified and samples from different organs (liver, lungs, kidncys, spleen, intestine) were isolated for that histopathological examination.

The result showed sever pathological lesion such as granulomatous lesions in lungs and livers of infected animals, with depletion of white pulp of spleen and conjestion with degenerative changes seen in kidneys and intestine with infiltration of inflammatory cells. While the infected-treated animals show mild or no pathological lesion in their internal organs.

## INTRODUCTION

Tuberculosis is common and often deadly infectious disease caused by Mycobacterium, usually Mycobacterium Tuberculosis in humans[1].

A third of worlds population are thought to be infected with M. tuberculosis[2] and new infections occure at rate of about one per second[3].

The primary site of infection in the lungs is called the ghon focus, and in generally located in either the upper part of the lower lobe, or the lower part of the upper lobe, tuberculosis infection begins when the mycobacterium reach the pulmonary alveoli where they invade and replicate within the endosomes of alveolar macrophages[4], tuberculosis is classified as one of the granulmatous inflammatory conditions, macrophage, T cell, and B cell and fibroblast among the cells that aggregate to form agranuloma, with lymphocyte surrounding in infected macrophages, T lymphocyte was secrete cytokines such as interferon gamma, which activate macrophages to destroy the bacteria which they are infected[1] [4].

The infectious dose of tuberculosis is very low and inhaling less than 3 bacteria may cause an infection[5].

Effective tuberculosis treatment is difficults due to unusual stracture and chemical composition of mycobacterial cell wall which makes many antibiotics in effective and hinders the entry of drugs [6,7].

This microorganism have multidrug resistant.[8] There for the aim of this study is to demonstrate the effect of ethambutol on mice infected with Mycobecterim tuberculosis.

## MATERIALS AND METHODS

- Bacterial isolates: Mycobacterium tuberculosis were Isolates obtained from tuberculosis institute in Baghdad, the biochemical test were done to these Isolates to confirm their diagnosis and identification.(9)

**Culture media:**

Lowenstein Jensen media which is a special media for Mycobacterium tuberculosis prepared according to the production mannals.

**Determination of challenge dose:**

Prepration of bacterial suspension of the counting was made by using Mcfarlands tubes according to. (10)

**Drug used for treatment:**

Treatment commenced 4weeks after infection aperiod which corresponds to the peak in primary lesion, using ethambutol which prepared in 50% sucrose and estimated according to.(11)

**Experimental design:**

Thirty of white swisrland mice, 6-8 weeks age were randomly divided equally into 3 groups and each group co 10 animals treated as the following:

1- The 1st group (group of infection) were inoculated intra peritonealy with 0.1 ml of bacterial suspension contain $1x10^8$ CFU of Mycobacterium tuberculosis

2- The 2nd group (group of treatment): Were treated as the 1st group but were given 0.1 ml (5mg/kg body weight ethambutol orally daily after 30 day post infection.

3- The 3rd group: were maintained as acontrol and were inoculated with 1ml of sterial normal saline intraperitonily all animals of all groups were sacrifed after 60 days post infection.

Post mortem examination done to all groups recording any gross lesion and pieces (1x1x1cm) from internal organs (liver, lungs, kidneys, spleen, intestine) were isolated fixed in 10% normal buffered formaline for 72hrs. then used the routen procedure for histopathogical section preparation according to (12)

# RESULTS

**- Pathological changes:**

Conjestion of the most examined organs the main gross lesion in both infected and treated groups with multiple focal granulomatous lesion in the liver and lungs of infected non treated group.

**Histopathological examination:**

Infected-non treated group:

- **Liver:** multiple focal granulomatous lesion in liver parenchyma especially around of central vein consist mononuclear cell aggregation and vacular degeneration of hepatocyte (figure. 1) also showed multifocal coagulative necrosis with infiltration of inflammatory cells.
- **Lung:** large granulmatous lesion consist of macrophages aggregation in lung parenchyma (figure 2).
- **Spleen** showed depletion of white pulp with conjestion of red pulp(figure 3)

**Kidney:** area of conjestion of blood vessel with infiltration of inflammatory cells in their lumen (figure4) and showed together with acute cellular degeneration charactrized by vaculation of cytoplasm of epithelial cells lining renal tubule.

**Intestine:** heavy inflittration of inflammatory cells (usually lymphocyte) between mucosal glands with sever conjestion of blood vessels.

**2- Infected-Treated animals:**

**- Liver:** histopathogical section showed conjestion of central vein with mononuclear cell in their lumen (figure 5) also proliferation of kupffer cell (figure 6)

**- Lung:** The lung showed no pathological lesion except conjestion of blood vessel with few inflammatory cell in their lumen (figure 7) and inter alveolar septa (figure8)

**- Spleen:** clear histopathological lesion was showed.

**- Kidney** there is moderate cell degeneration of epithelial cells lining renal tubule with neutrophil infiltration (figure 9)

**- Intestine** microscopic section revealed few inflammatory cells between mucosal glands (figure10).

**The table showed the mean and standard error of both infected and treated animals, and the efficacy of ethambutol in treatment.**

| No. | First group (infected animal) | Second group (treated animal) |
|---|---|---|
| 1. | 3(+++) | 1(+) |
| 2. | 3(+++) | 1(+) |
| 3. | 3(+++) | 1(+) |
| 4. | 2(+++) | 0(-) |
| 5. | 3 (+++) | 1(+) |
| 6. | 3 (+++) | 0(-) |
| 7. | 3 (+++) | 0(-) |
| 8. | 2 (+++) | 0(-) |
| 9. | 3 (+++) | 1(1) |
| 10. | 3 (+++) | 1(1) |

| Animal | Mean | N | Std. Deviation | Std. error Mean | t-test | Sig |
|---|---|---|---|---|---|---|
| Infected | 2.8000 | 10 | 0.42164 | 0.13333 | 15.057 | Hs |
| treated | 0.5000 | 10 | 0.52705 | 0.16667 | | |

Hs= 0.05 تعني ان هناك اختلافات معنوية عالية عند مستوى اختبار

+++ =3 sever lesion

++ = 2 medium

+ =1 less effect lesion (simple l

esion)

-=0 no lesion

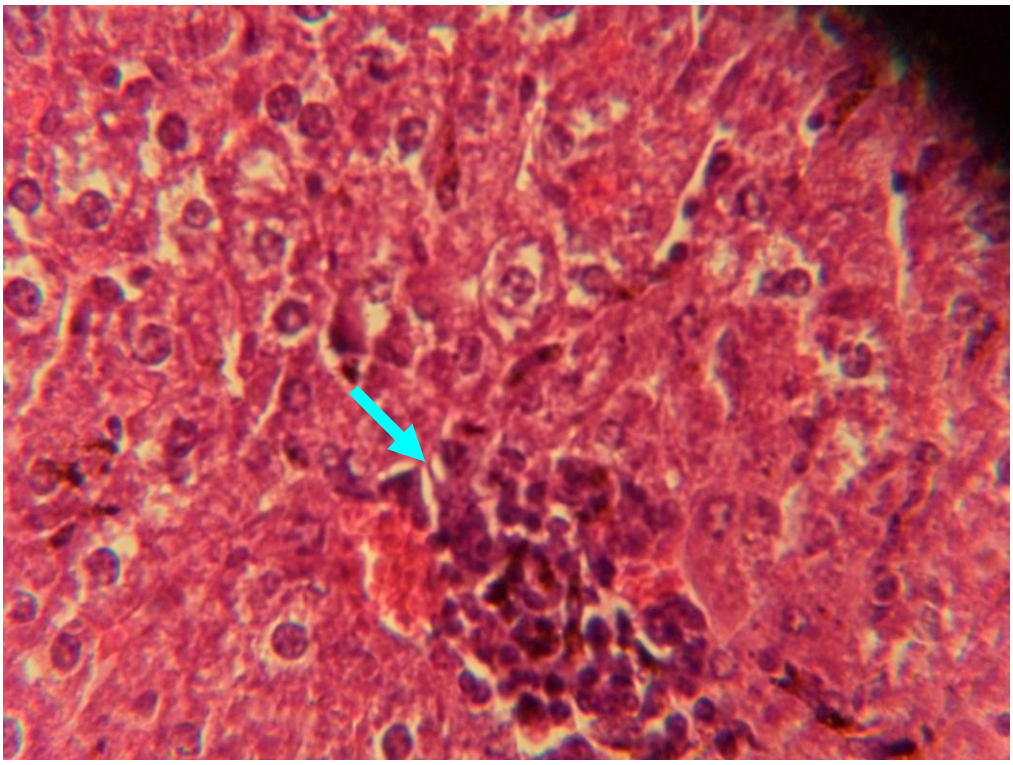

Fig. (1): Histological section in liver of of infected non-treated group showed multiple focal granulamatous lesion in liver parenchyma especially around central vein consist of aggregation of mononuclear cells(⟶) (H & E 10X)..

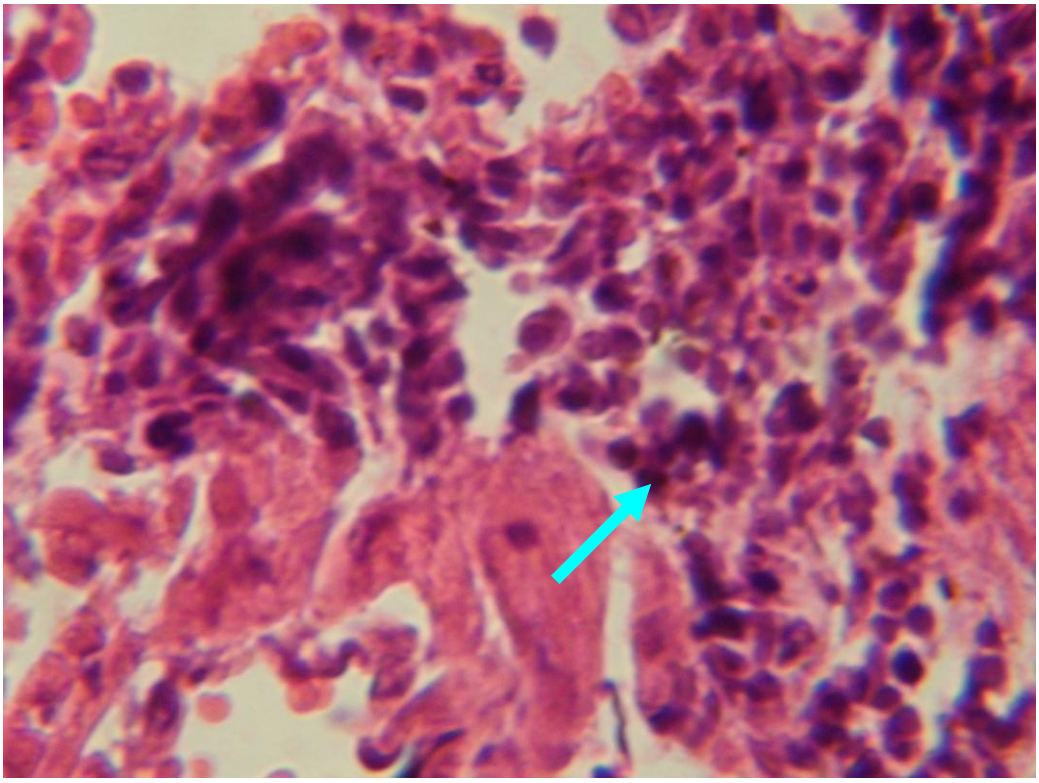

Fig. (2): Histological section in lung of of infected non-treated group showed large granul matous lesion consist of aggregation of macrophages in lung parenchyma.
(⟶) (H & E 40X)

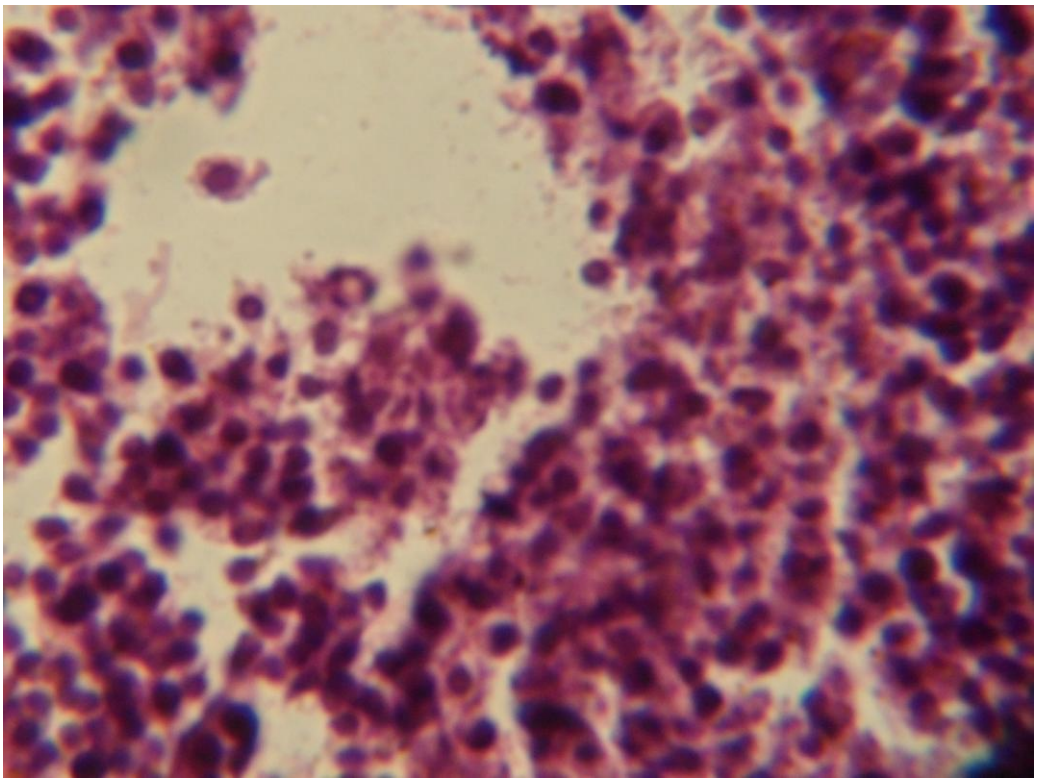

Fig. (3): Histological section in spleen of infected-non treated group showed depletion of white pulp with conjestion of red pulp. (⟶) (H & E 40X)

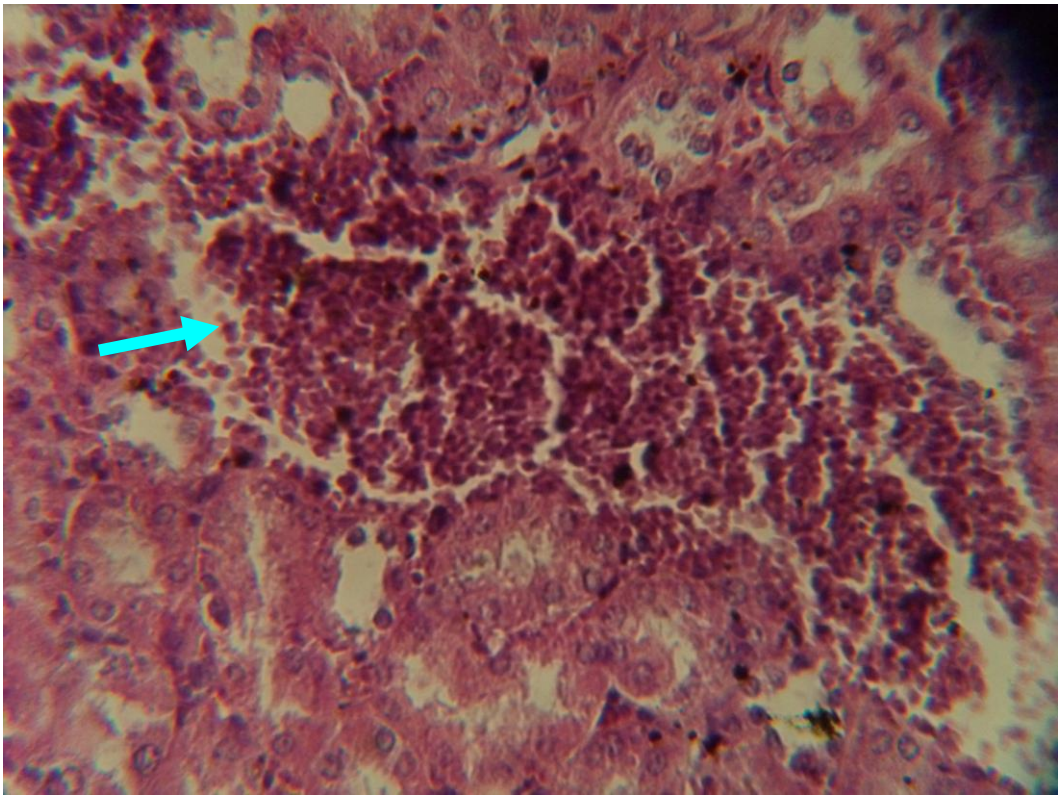

Fig.(4): Histological section in kidney of infected-non treated group showed conjestion of blood vessel with infiltration of inflammatory cell in their lumen. (⟶) (H & E 10X)

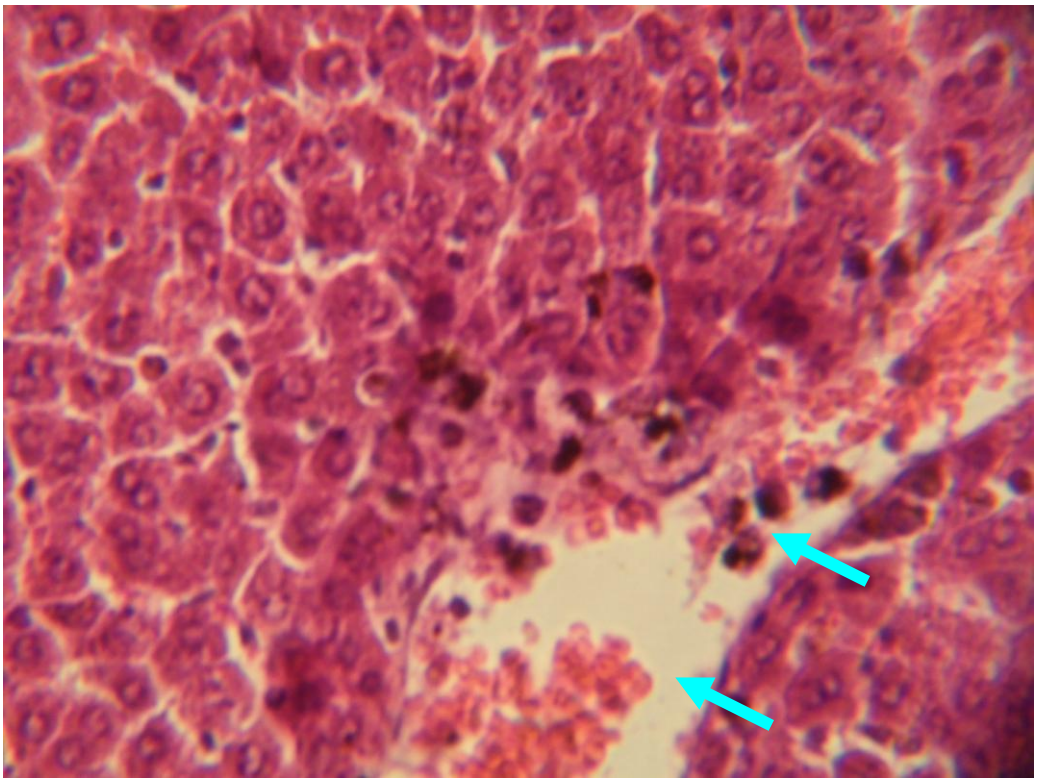

Fig. (5): Histological section in liver of treatment group with thambutol showed conjestion of central vein ( ⟶ ) with mononuclear cell in their lumen.
(⟶) (H & E 40X)

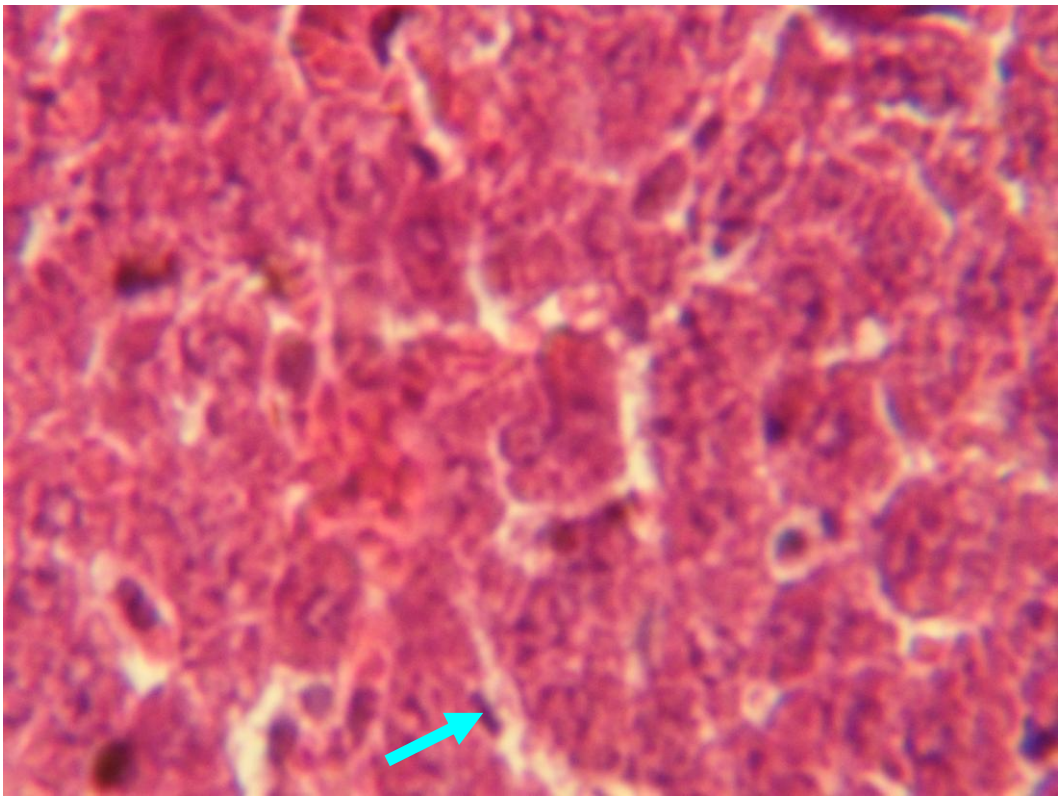

Fig.(6): Histological section in liver of treatment group with ethambutol show proliferation of kupffer cell. (⟶) (H & E 40X)

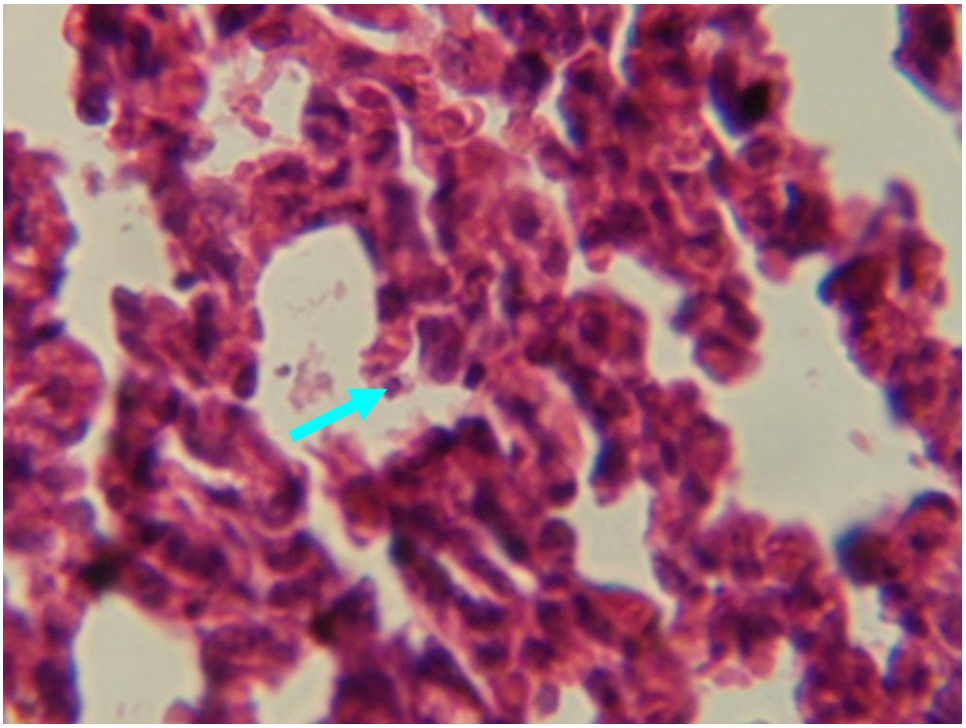

Fig.(7): Histological section of lung of treatment group revealed but conjestion of blood vessel with few inflamtory cell in their lumen. (⟶) (H & E 40X)

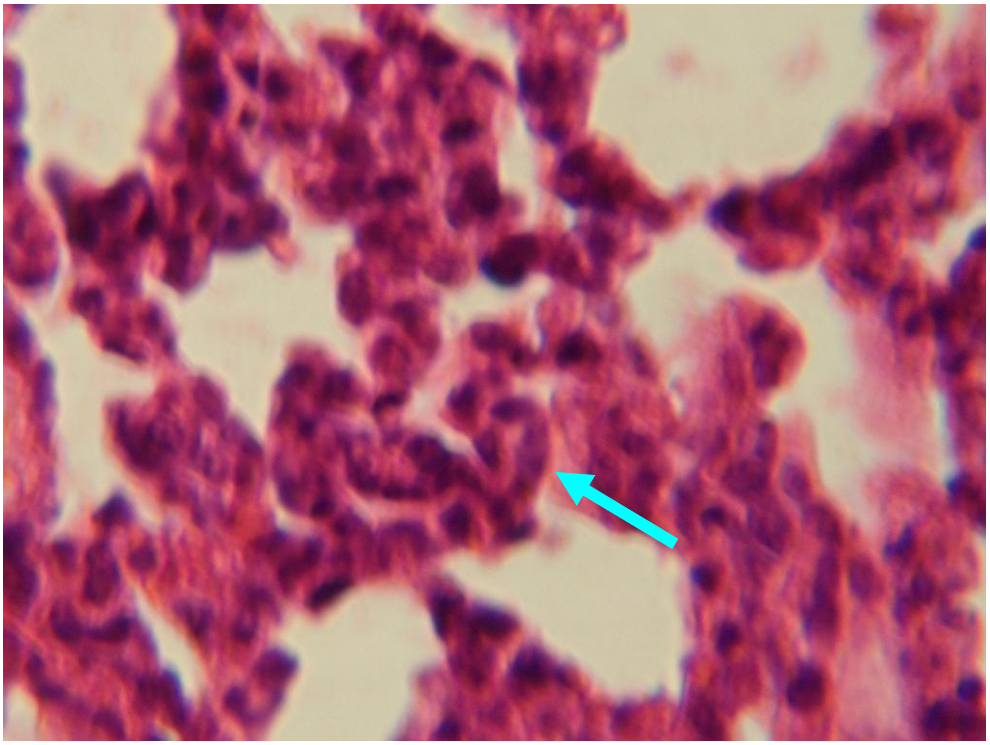

Fig.(8): Histological section of lung of treatment group showed aggregation of mononuclear cell in the interalveolar septa. (⟶ ) (H & E 40X)

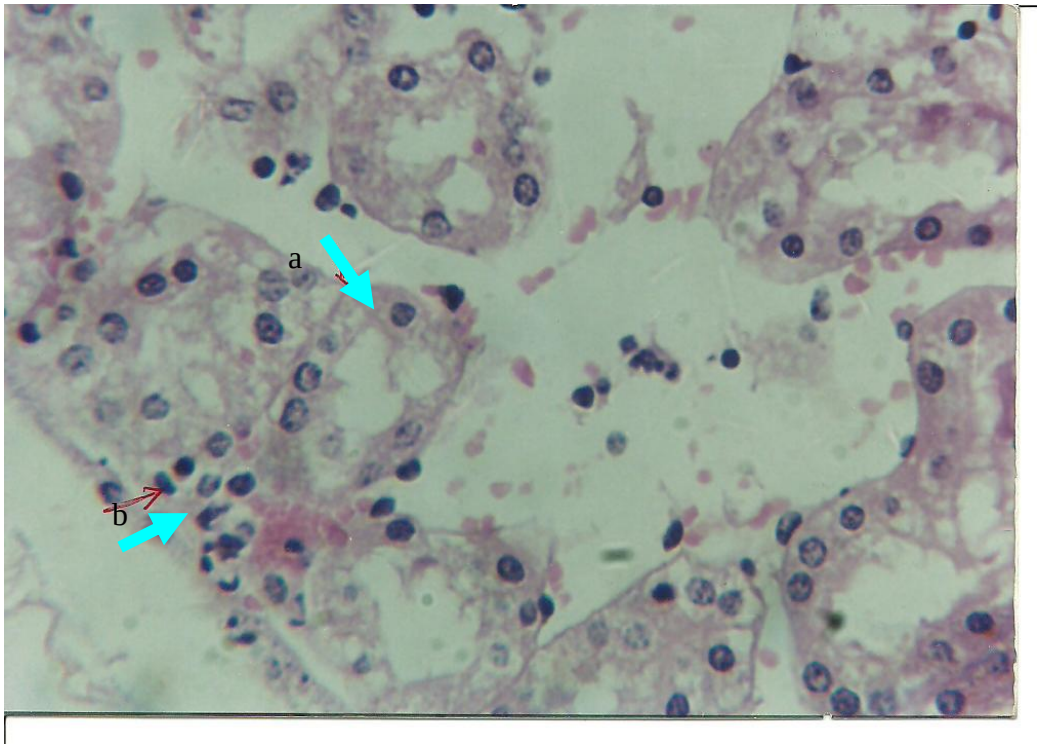


Fig.(9): Histological section of kidney of treatment group revealed cellular degeneration of epithelial cell lining renal tubule.( ⟶ a) and neutrophil infiltration in the interstitial tissue ( ⟶ b )(H & E 40X)

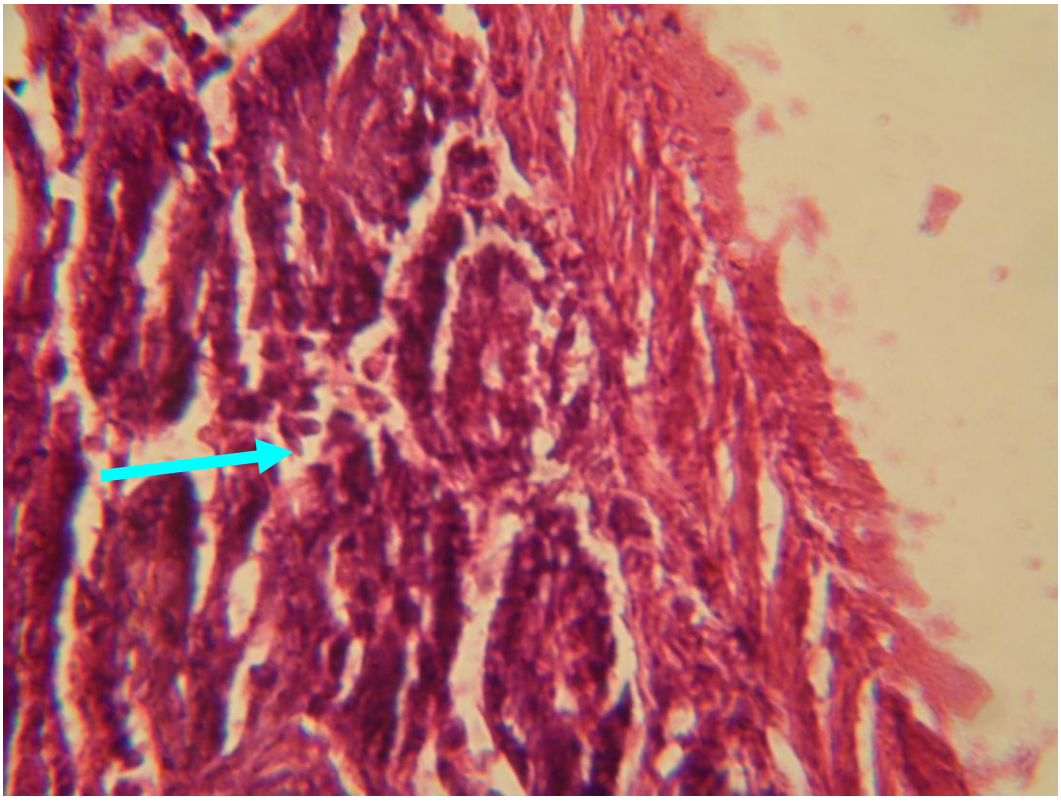

Fig. (10): Histological section of intestine of treatment group reveled few inflammatory cell between mucosal glands. ( ⟶ ) (H & E 10X)

## DISCUSSION

In this study we have shown that intra peritoneal infection does result in chronic TB infection in mice organs similar to that observed during low dose aerosol infection mentioned by.(13)

The present study revealed that the main histopathological in the examined lungs and livers of inflected animals were granuloma and this observation agreed with.(14)

The absence of necrosis and langhans type cells makes the tuberculosis granulomas of mice histologicaly different from those arising in guinea pigs, rabbits and humans and this result supported the investigation that mentioned by (15), these differential features have been related to the stronger immunological response elicited in mice, which consequently tend to sustain lesser degrees of systemic dissemination of mycobacterial infection than guinea pigs or rabbits,(16).(17).

The Mycobacterion tuberculosis spread through the blood stream to other tissues and organs and so all parts of body can be affected by the disease. ( (18) and this agree with our result, that the Micro Organsim reach to different organs (lungs, liver, kidneys, intestine and spleen).

Virulence is the measure of pathogenicity of amicroorgansim as determined by it, ability to invade host tissue and produce disease, and after thirty days of infection bacilli had disseminated resulting in microscopically visible lesions in liver, spleen, lungs, and these result supported by the observation.(19)

Tuberculosis is classified as one of the granulomatons inflammatory conditions, macrophage, T cell, B cell and fibroblasts are among the cells that aggregate to form granuloma with lymphocytes surrounding the inflected macrophages, T lymphocyte secrete cytokines such as interferon gamma which activate macrophages to destroy bacteria.(20)

The treatment with ethambutol resulted in significant reduction in pathological lesion in different organs and our result is agreed with (21) Who use aguinea pig as amodel of infection.

Ethambutol inhibits arabinosyl transferase-an enzyme that is important for the synthesis of the mycobacterial arabinogalactan cell wall.(22)

# دراسة بعض التغيرات المرضية لمجاميع الفئران المصابة بجرثومة السل البشري والمعالجة باستعمال عقار الايثامبيتول

سهير الكتبي

كلية الصيدلة ،جامعة بغداد،بغداد،العراق

## الخلاصة

لغرض دراسة التغيرات المرضية النسيجية ومعرفة تاثير عقار الايثامبيتول على اعضاء الفئران المصابة بجرثومة السل البشري mycobactoriam tuberculosis ، استخدم 30 من الفئران السويسرية البيضاء ومن كلا الجنسين والتي تتراوح اعمارها بين 6-8 اسبوع وباوزان 20-25غم، قسمت هذه الحيوانات عشوائيا الى ثلاث مجاميع ضمت كل منها 10 حيوانات:

المجموعة الاولى: (مجموعة الاصابة) تم اصابتها بجرثومة السل البشري بطريقة الحقن داخل الخلب بجرعة 0.1مل من العالق الجرثومي الحاوي على $10x1^{8}$ Cfu من الجراثيم.

المجموعة الثانية: وهي مجموعة (الاصابة والعلاج ) تم اصابتها كما في المجموعة الاولى وبعد مرور 30 يوم على الاصابة عولجت بعقار الايثامبيتول بجرعة 5 ملغم/كغم يومياً عن طريق التجريع بالفم.

المجموعة الثالثة: عدت بوصفها مجموعة سيطرة للمجاميع المصابة حيث حقنت بـ 1 مل من المحلول الفسلجي المعقم داخل الخلب.

وبعد مرور 60 يوماً من الاصابة تم قتل حيوانات المجاميع الثلاث واخذ عينات من الاعضاء الداخلية (الكبد، الرئتين، الكلى، الطحال، الامعاء) لغرض دراسة التغيرات المرضية.

اظهرت النتائج حدوث افات مرضية شديدة تميزت بالافات الحبيبية المنتشرة في اكباد ورئات الحيوانات المصابة اضافة للاحتقان والتغيرات للتنكسية وارتشاح الخلايا الالتهابية في الطحال والكلى والامعاء.

اما مجموعة الاصابة والعلاج فقد اظهرت تغيرات وافات مرضية تراوحت بين الشدة الاقل والشفاء التام.

## REFERENCES

1- Kumar, V.; Abbas, A.; Fausto, N. and Mitchell, R. (2007), Robbins Basic pathology (8thed.) Saunders Elsevier p.516-522.

2- Jasmer, R. M.; Nahid, P. and Hopewell, P. (2002) "clinical practice, latent tuberculosis" N. Eng. J. med 347(23): 18 60-66.

3- World health organization (2009), Tuberculosis. (http://who.int/media center/factsheets/fs204/en/index.html.)

4- Houben, E.; Nguyen, L. and pieters, J. (2006).

"Interaction of pathogenic mycobacteria W.Th the host immune system. Curr. Opin. microbiol 9(1). 76-85.

5- Nicas, M.; Nazaroff, W. W. and Hubbard, A. (2005) "Toward understanding the risk of secondary air borne infection: emission of respirable pathogens" J. Environ Hyg 2(3): 143-54.

6- Brennan, p.j. and Nikaido, H. (1995) "The envelope of mycobacteria". Annu. Rev. Biochem. 64:29-63.

7- Acharya, p.v. and Goldman, D.S.(1970) "Chemical composition of the cell wall of the H37Ra strain of Mycobacterium tuberculosis". J. Bacteriol 102(3): 733-39.

8- Brien, R. (1994) "Drug-resistant tuberculosis; etiology, management and prevention". Semin Respir infect 9(2): 104-112.

9- Quinn, P.J.; carter, M.E.; Markey, B. and carter, G. R. (2004) clinical vet. Microbiol. 6th ed. Mosby animp. Wolf, London,.

10- Balasubramanian, V.; Guo-zhi, W.; Wiegeshaus, E. and Smith, D. (1992). "Virulence of Mycobacterium Tuberculosis for guineapigs :a quantitative modification of the assay developed by mitchison. In J. Tuberc. And lung Dis., 73: 268-272.

11- Smith, D.W.; Balasubramanian, V. and wiegeshaus, E. (1991), "Aguinea pig model of experimental airborne tuberculosis for evaluation of the response to chemotherapy: the effect on bacilli in the initial phase of treatment" tuberde 72:223-231.

12- Luna, L.G. (1968). Manual of histology stuining methods of the armed forces institute of pathology 3rd ed. Mcgrow-Hill, Book company. New York.

13- Cardona, p.j.; Cooper, A.; Luguin, M. (1999) "the intravenous model of murine tuberculosis in less pathogenic than the aerogenic model owing to a more rapid induction of systemic immunity. Scand J immunol 49:362-366.

14- Cardona, p.j.; LLatjos, R; Gordillo, J., Diazej ; Ojanguren, I; Ariza, A. and Ausina, A.(2000) "evolution of granuloma in lungs of mice infected aeroginically with Mycoboterium tuberculosis" scand J. immunol 52: 156-163.

15- Lefford, M.j. (1984) "disease in mice and rats In: kubica. GP. The mycobacteria: Asource book Marcel Dekker Inc. New York: P:947-977.

16- Orme, I.M. (1996). The immune response to tuberculosis in animal models. Tuberculosis Boston: little. Brown: 269-280.

17- Converse, p.j. (1996) "cavitary tuberculosis produced in rabbits by aerosolized virulent tubercle bacilli. Infect immune. 64: 4776-87.

18- Agarwal, R.; Malhotra, p., Awasthi, A.; Kakkar, N and Gupta, D. (2005) "Tuberculosis dilated cardiomyopathy: an under-recognized entity?" BMC infect. Dis. 5(1):29.

19- Gopinath, S.; palanisamy; Smith, E.; Crystal, A. S.; Diane, J. O.; Ian; M.O and Randall, J.B. (2009) "Disseminated disease severity as ameasure of virulence of Mycobacterium tuberculosis in guineapig model. Tuberculosis 8(4): 295-306.

20- Kaufmann S(2002). "Protection against tuberculosis: cytokines, T cells and macrophages" Rheum Dis. 61 Suppl 2: 54-58.

21- Dickinson, J.M and Mitchison, D.A. (1976) "Bactericidal activity in vitro and in guinea pig of isoniazid, rifampicin and ethambutol. Tubercle 57:251-258.

22- Richard Philadelphia illustrated Reviews 3rd edition, D. Howland and Mary J. Mycek, (2006),Lippincott's, Lippincott's Williams and wilkins, p.399.