

An Immune Mediated Pathology And Tissue Immune Responses Induced By Human Tuberculus Cryoglobulin In A Murine Models

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Abstract

Serum cryoglobulin were separated , characterized and partially purified from human pulmonary tuberculus patients. Three graded concentrations were made as;0.2,0.3,and 0.4 mg/ml .Four groups of mice, each of five were given 0.25ml. intramuscular in left and 0,25ml., in the right thigh to each animal in each group .the protocol consist of three successive doses from each concentration to each group in a week a part trend followed by one week leave .At the end of the protocol ,animals were sacrificed as well as the tissue blocks were collected, sectioned and processed for staining with H&E by the aim of tracing immune responses at tissue level .The immune response events were found as; Hyper-plasia of showed bronchus associated lymphoid tissue ,hyperplasia of epithelial cells of the bronchus walls, hyperplasia of macrophages in the alveolar spaces and accumulation of macrophages in the bronchial walls of the lungs .Cardiac muscles showed signs of carditis indicating type II hypersensitivity .Kidneys have shown inflammatory events indicating nephritis may be of serum sickness origin and edema can be of Arthus reaction, hypersensitivity type III. Liver sections were showing macrophage between blood vessels as pre-vascular kuff indicating delayed type hypersensitivity type IV. While splenic tissue sections were showing hypoplasia of lymphoid cells in the white pulp and /or depletion which may be attributed to the action of the regulatory T cells to the white pulp lymphoid cells. The tissue nature do affect the type of response as well as the intensity of the tissue responses were concentration dependent. Human cryoglobulin from tuberculus patients were found pathogenic for mice and the pathology was immune mediated as; Hypersensitivity types II,III, and IV as well as granuloma. The intensity of the responses were concentration dependent.

Key Words: Cryoglobulin, granuloma, hyperplasia ,hyoplasia ,hypersensitivity, mucosal response.

التغيرات المرضية المناعية والاستجابة المناعية النسيجية المستحدثة بواسطة الكلوبولين البارد

المعزول من اصابات السل الرئوي في الفئران

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المستخلص

تنقية البروتين المسمى الكلوبولين البارد من فصل ثم عزل من المرضى المصابين بمرض داء السل الرئوي ، حيث تم استخدام ثلاثة تراكيز مختلفة وهي (0.2,0.3,0.4). تم استخدام اربعة مجاميع من الفئران وكل مجموعة تحتوي على خمسة حيوانات واعطي كل حيوان في كل مجموعة 0.25 مل في الفخذ الايسر والايمن من بروتين الكلوبولين البارد. تضمنت الدراسة استخدام ثلاثة جرع من كل تركيز لكل مجموعة ولمدة اسبوع متبوعه باسبوع راحة خلال مدة الدراسة وبالغلة اربعة اسابيع .وفي نهاية التجربة تم قتل وتم جمع العينات وقطعت وتم صبغها بصبغة الايوسين والهيما توكسلين والهدف من ذلك هو التحري عن الاستجابة المناعية على مستوى النسيج وكانت الاستجابة المناعية كالآتي :فرط التنسج للخلايا الطلائية المبطنة لجدار القصبات الهوائية مع تواجد خلايا لمفاوية . اظهر نسيج العضلات القلبية وجود علامات التهاب شغاف القلب وكان المؤشر زيادة التحسس النوع الثاني . كما اظهرت نتائج الفحص النسيجي لمقاطع الكلية وجود علامات التهابية تمثلت بوجود التهاب الكلية لاحتمالية وجود Serum sickness origin وذمة تشير الى وجود تفاعل ارثس (زيادة فرط التحسس النوع الثالث). في حين اظهرت المقاطع النسيجية للكبد وجود خلايا التهابية بين الاوعية الدموية والتي تمثلت بوجود pre-vascular kuff والتي تشير الى فرط التحسس المتأخر الرابع. كما بينت النتائج في نسيج الطحال حدوث قلة التنسج والمتمثل بالخلايا اللمفاوية في اللب الابيض والذي يعزى الى فعالية الخلايا التائية المنظمة الى الخلايا اللمفاوية للابيض. طبيعة النسيج اظهر تاثير نوع الاستجابة بالاضافة الى شدة استجابة النسيج بالاعتماد على التركيز. بالاعتماد على النتائج اعلاه اظهرت بان بروتين الكلوبولين البارد المعزول من المرضى المصابين بالسل الرئوي يسبب امراضية في الفئران المختبرية وتمثلت الامراضية باحداث تغيرات مناعية كالآتي زيادة التحسس النوع الثاني والثالث والرابع بالاضافة الى حدوث الورم الحبيبي وكانت شدة الامراضية تعتمد على الكلوبولين البارد.

الكلمات المفتاحية: الكلوبولين البارد ، الورم الحبيبي ، فرط التنسج ، قلة التنسج، فرط الحساسية ، الاستجابة المخاطية.

Introduction

ImmunoglobulinG3RF generates cryoglobulins both in an in-vitro and in-vivo systems and that some cryoglobulins were found highly pathogenic in murine model[14].In transgenic mice implanted with hybridoma secreting 6-19IgG3and anti

IgG2aRF with cryoglobulin activity. Such cryoglobulin developed an acute glomerulo-nephritis and cutaneous leukocytoclastic vasculitis. Unique combination of heavy & light chains are so important for the expression of the pathogenic activity of IgG2 cryoglobulin[1].Thymic stromalin lymphopoieten transgenic mice develop cryoglobulin and hepatitis in which liver injury was mild to moderate with minimal to mild fibrosis and deposition of immunoglobulin around the portal tract [2].Shnawa and AlSerhan[12] have shown the pathogenic potentials of the typhoid specific cryoglobulin from man and rabbit in a lapin model. The objective of the present work was first to prove the pathogenic potentials of human tuberculus cryoglobulin and second to map immune response at tissue level in mice.

Materials And Methods

Cryoglobulins:

The human cryoglobulin associated with pulmonary tuberculosis were separated, characterized ,partially purified and quantified as in [4].Then rated to 0.2,0.3,and 0.4 mg/ml.

Immunization [12] :

Four groups of white mice each of five were the test immune system .They were adapted for housing conditions for one week, then were immunized as follows;

Group I: 0.2 mg/ml., Group II : 0.3 mg /ml., Group III : 0.4 mg/ml. Group IV: 0.0 mg/ml.

The protocol was through intramuscular injection of 0.5 ml. to each mice in each group for each concentration .Of which 0.25 in the left and 0.25ml in the right thigh muscle in a week a part followed by one week leave.

Tissue Sampling And Processing:

Mice in each group were sacrificed at the end of the immunization period .Tissue block of 1 cm. were taken from; lungs, liver, spleen ,heart and kidneys. Tissues were processed ,sectioned and stained as in [7].

Results and Discussion :

The lung tissue sections of mice primed with 0.2 mg/ml. human tuberculus cryoglobulin has showing proliferation of alveolar macrophage in the alveolar spaces as well as hyperplasia of bronchial associated lymphoid tissues. While ,at 0.3 mg/ml. tissue sections showed epithelial cell hpyerplasia, mononuclear cell hyperplasia and mononuclear cell infiltration in the bronchial wall and alveolar space. At 0.4 mg/ml., however, the lung sections shows hyperplasia of epithelial cell and mononuclear cell infiltration in the wall of bronchiole with proliferation of alveolar macrophages(Figures 1,2,3)., while The liver sections of mice inoculated with 0.2 mg./ml .and 0.3 mg/ml. human tuberculus cryoglobulins shows mononuclear cell aggregation in the liver parenchyma and around vessels. Sections of 0.4 mg/ml. ,primed mice ,liver shows fatty cell degeneration in hepatocytes and granulomma formation that consist of macrophages and neutrophils (4,5,6). On the other hand, Splenic sections of mice primed with 0.3 mg/ml., shows white pulp lymphoid cell hypoplasia. While the 0.4 mg/ml., primed mice ,splenic sections shows white pulp depletion of lymphoid cells(Figures 7,8). Kidney tissue sections of 0.2 mg./ml.,primed mice showed normal

tissue sttings. While the sections of 0.3mg./ml., primed mice has shown atrophy in the glomerular tifts and vascular degeneration of the epithelial cells forming the renal tubules. The tissue sections of the mice primed with 0.4mg./ml., have shown neutrophilic and macrophagic infiltration between renal tubules with edema and acute cell degeneration of the epithelium forming the tubules and epithelial cell enlargement to close the tubular lumen(Figures 9,10,11). The cardiac muscle tissue section of mice primed with 0.3 mg./ml., shows inflammatory cell infiltration and fragmentation of cardiac muscle fibers .At 0.4 mg/ml., primed mice heart tissue shows fragmentation edema of the muscle fibers(figures 12 and 13).

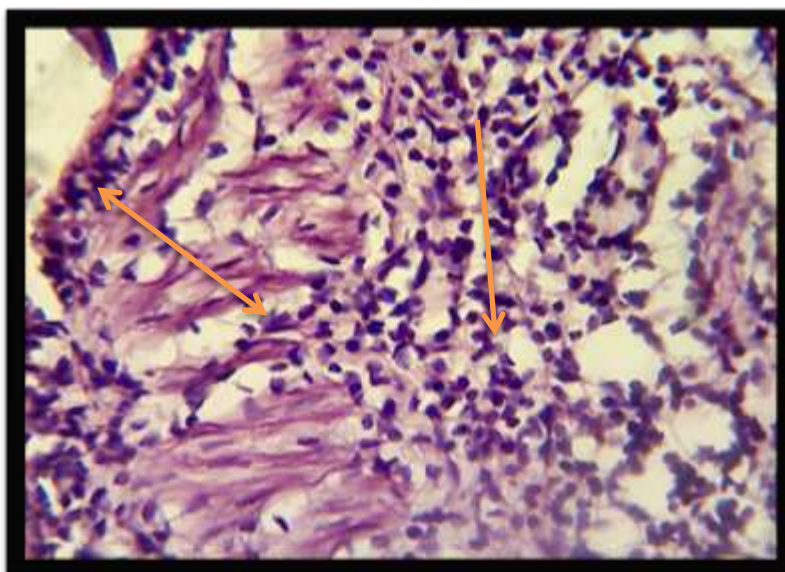


Fig1 :Histopathological section in the lung of animal at 26 days post-treatment with 0.3 of cryoglobulin shows hyperplasia of epithelial cells with hyperplasia \longleftrightarrow and mononuclear cells infiltration in wall of bronchi and alveolar spaces \longrightarrow (H&E stain 40X).

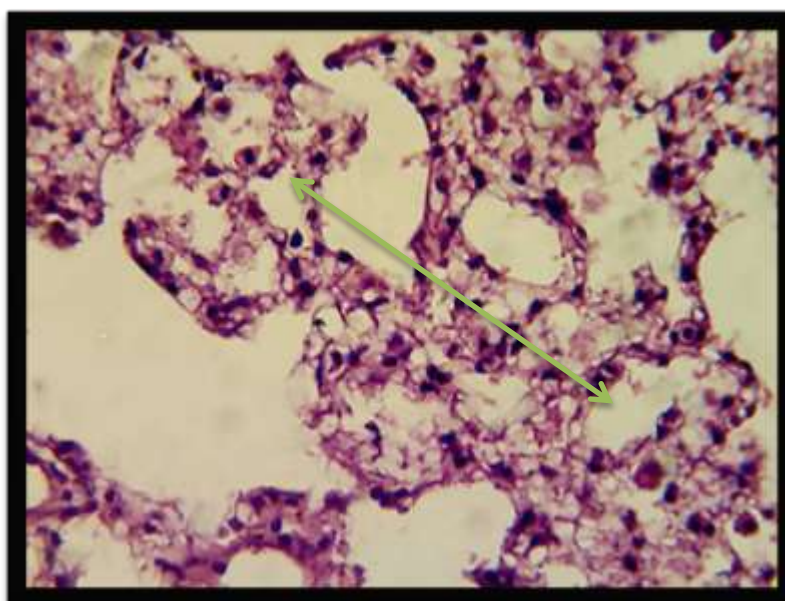


Fig2:Histopathological section in the lung of animal at 26 days post-treatment with 0.2 shows proliferation of alveolar macrophages in alveolar spaces \longleftrightarrow (H&E stain 40X)

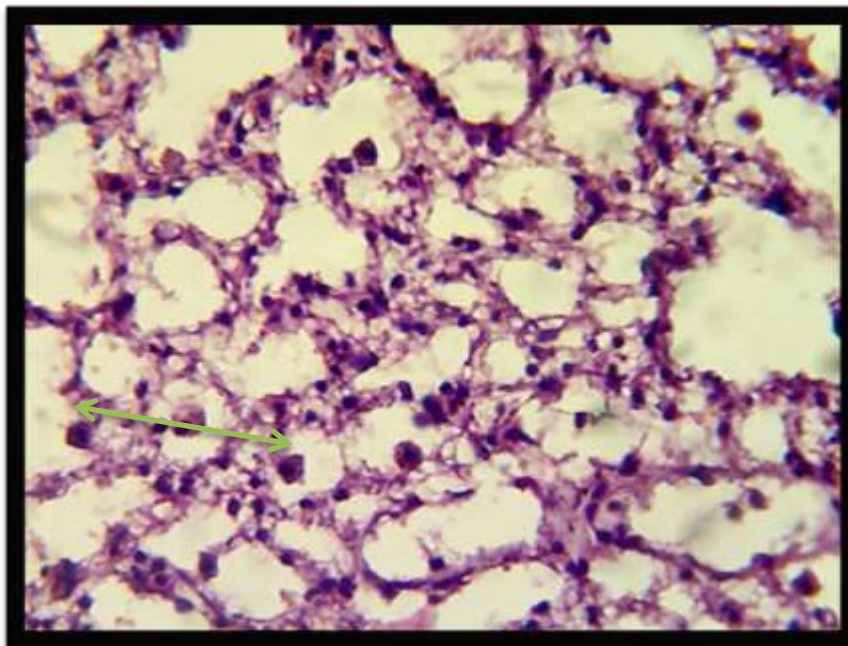


Fig3:Histopathological section in the lung of animal at 26 days post-treatment with 0.4 shows proliferation of alveolar macrophages in alveolar spaces \longleftrightarrow (H&E stain 40X).

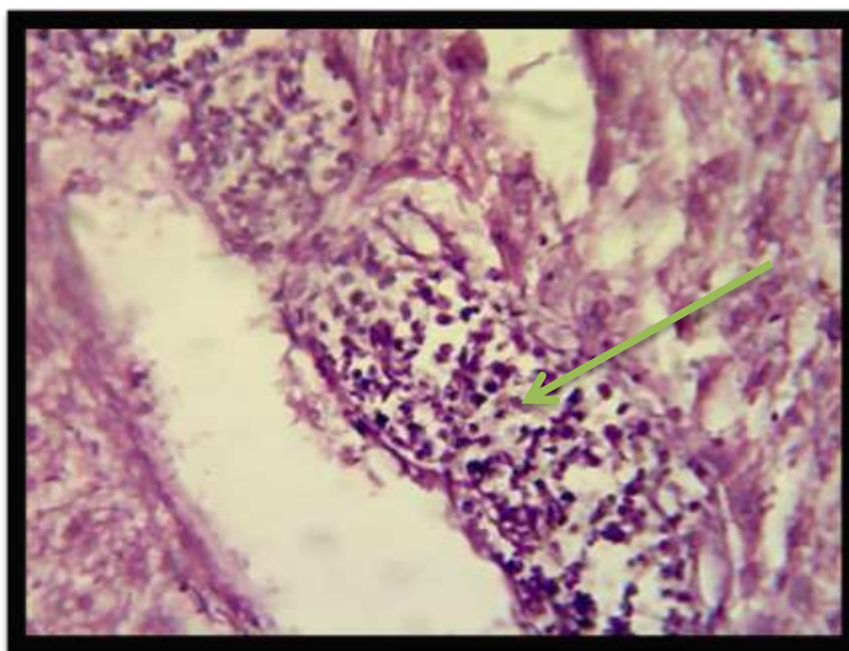


Fig4:Histopathological section in the liver of animal at 26 days post-treatment with 0.3 of cryoglobulin shows mononuclear cells aggregation around blood vessels \longrightarrow (H&E stain 40X).

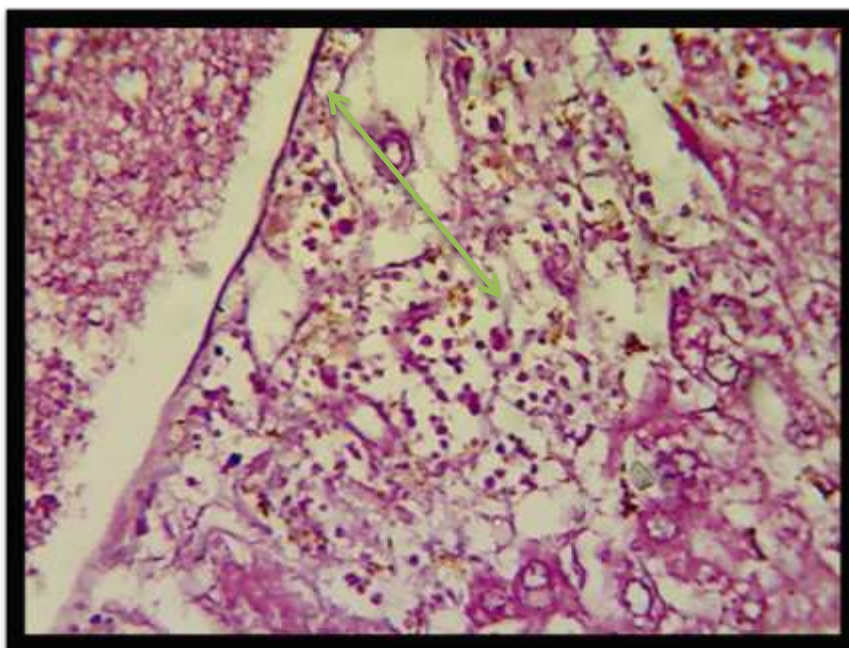


Fig5: Histopathological section in the liver of animal at 26 days post-treatment with 0.2 shows mononuclear cells aggregation in liver parenchma and around blood vessels \longleftrightarrow (H&E stain 40X).

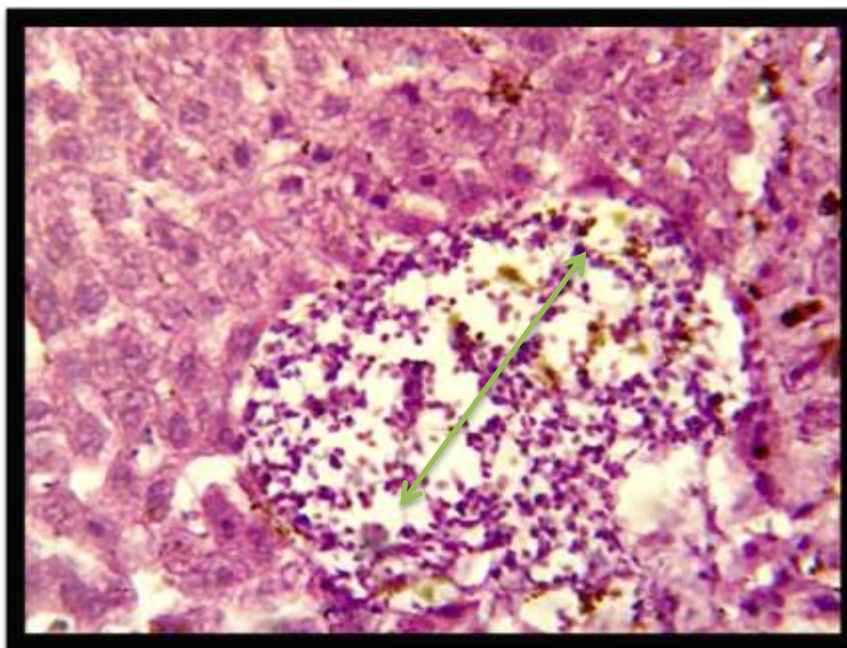


Fig6: Histopathological section in the liver of animal at 26 days post-treatment with 0.4 shows granulomatous lesions consisting from aggregation of macrophages and neutrophils \longleftrightarrow (H&E stain 40X).

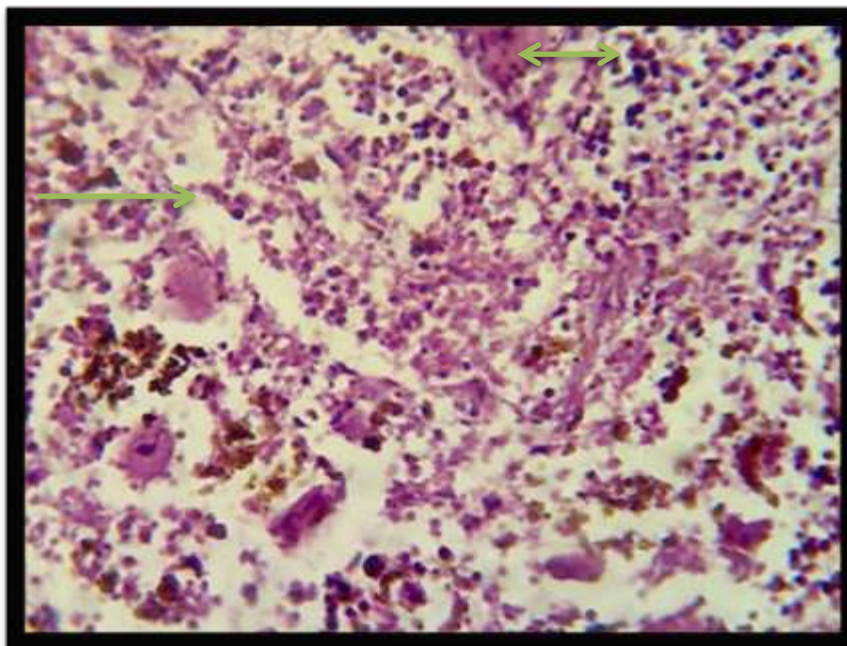


Fig7:Histopathological section in the spleen of animal at 26 days post-treatment with 0.3 of cryoglobulin shows atrophy of white pulp \longleftrightarrow and fibrin deposition \rightarrow (H&E stain 40X).

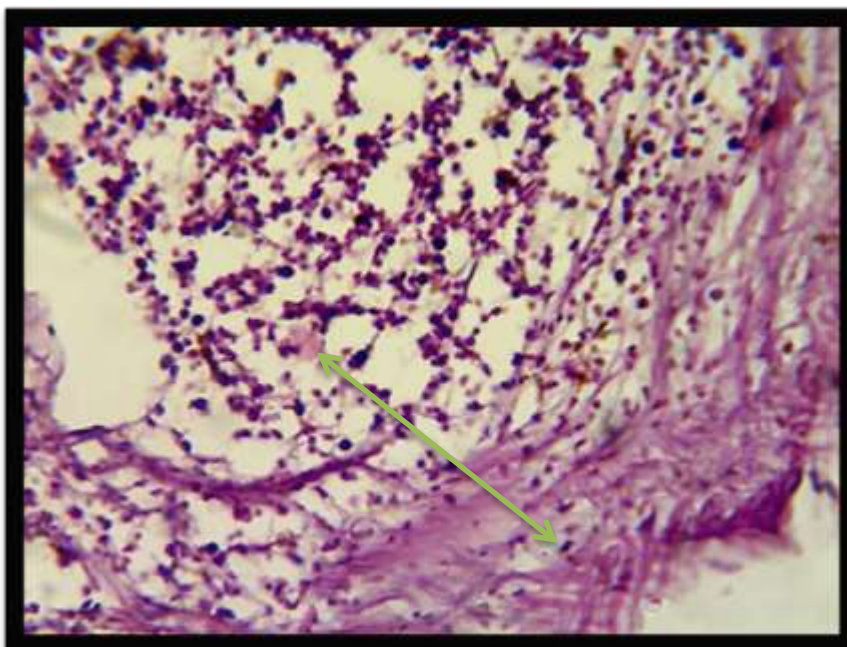


Fig8:Histopathological section in the spleen of animal at 26 days post-treatment with 0.4 shows depletion of white pulp \longleftrightarrow (H&E stain 40X) .

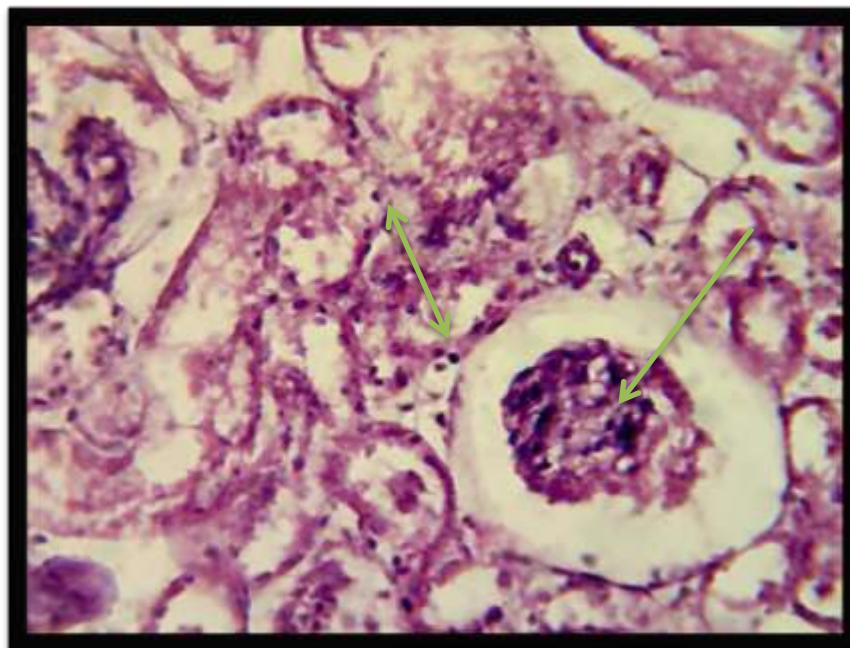


Fig9:Histopathological section in the kidney of animal at 26 days post-treatment with 0.3 of cryoglobulin shows atrophy of glomerular tufts → with vacuolar degeneration of epithelial cells of renal tubules ← (H&E stain 40X).

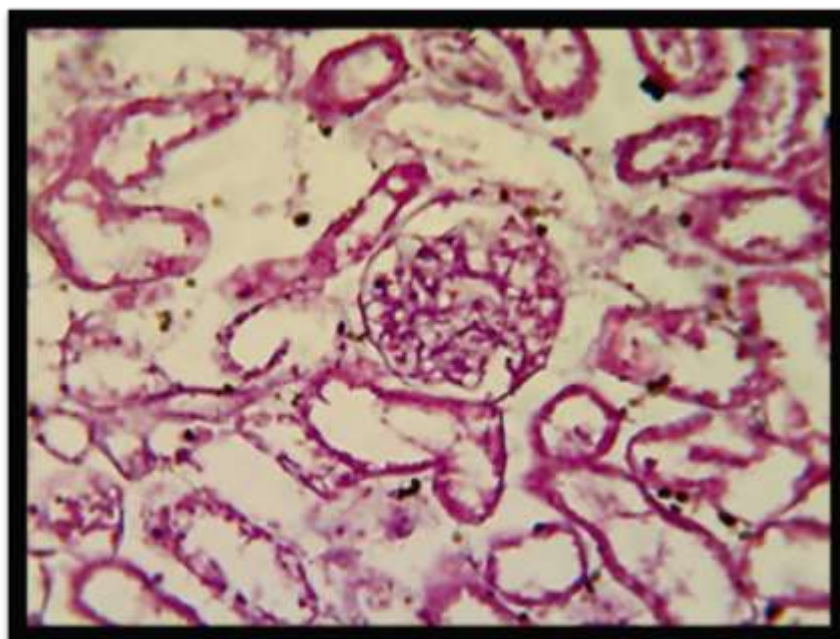


Fig10:Histopathological section in the kidney of animal at 26 days post-treatment with 0.2 shows no clear lesions (H&E stain 40X)

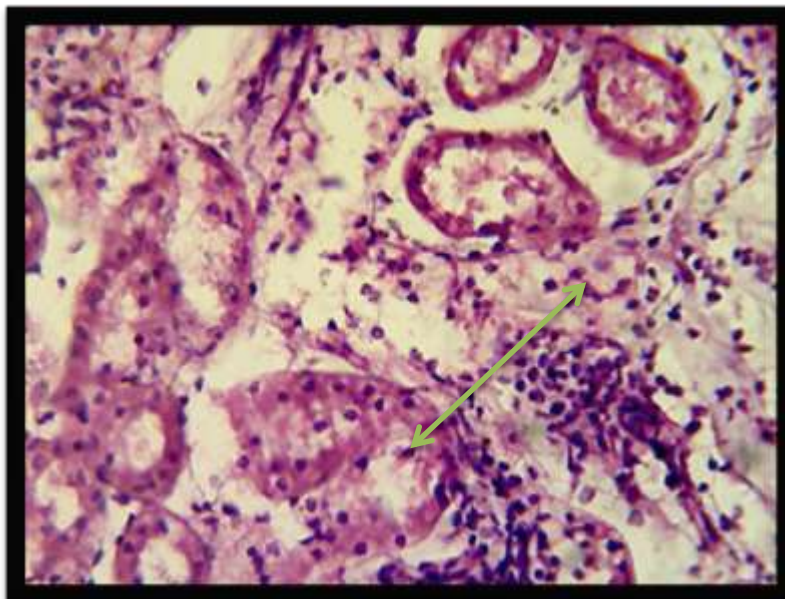


Fig11:Histopathological section in the kidney of animal at 26 days post-treatment with 0.4 shows inflammatory cells particularly neutrophils and macrophages between renal tubules (H&E stain 40X). ↔

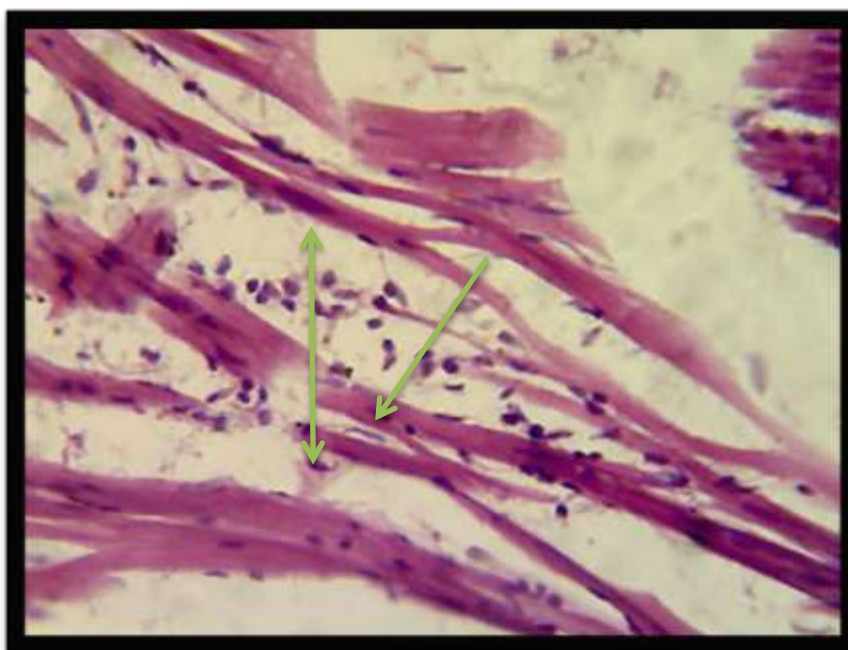


Fig12 : Histopathological section in the heart of animal at 26 days post-treatment with 0.3 of cryoglobulin shows inflammatory cells infiltration between cardiac muscle fiber ↔ and fragment of muscle fiber → (H&E stain 40X).

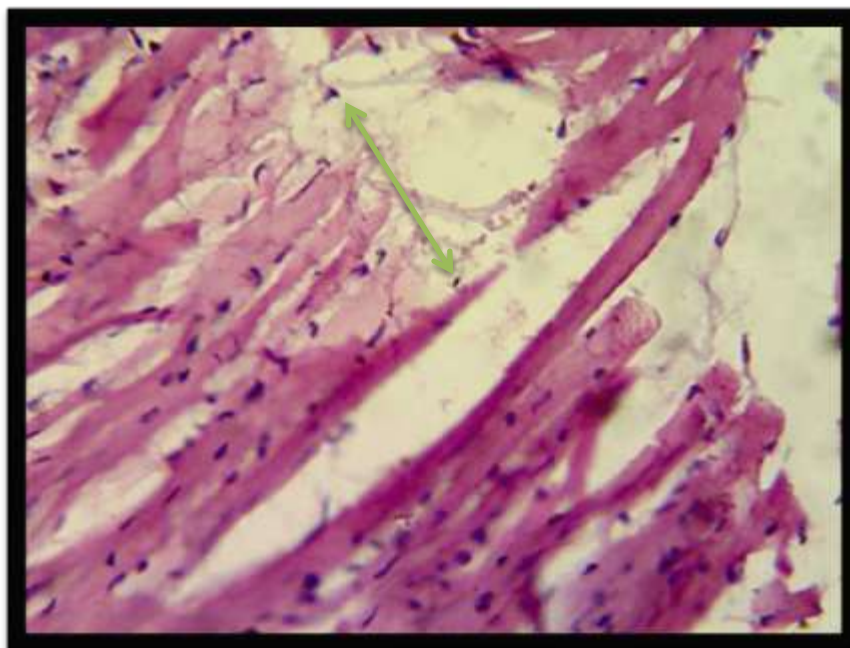


Fig13:Histopathological section in the heart of animal at 26 days post-treatment with 0.4 shows fragmentation of cardiac muscle fiber and edema between cardiac muscle fiber \longleftrightarrow (H&E stain 40X).

The human tuberculus cryoglobulin primed mice groups have shown various degrees of immune mediated tissue pathology in lungs, liver, spleen, kidneys and heart(Figures 1-13),and are being comparable to that of typhoid cryoglobulin in rabbits[12].The tissue immune response were traced in secondary phase of the immune response time curve. Male and associate 2006[5],have documented five distinctive immunologic characteristics of each tissue and these characters were evident on the microscopic screening of the tissue sections of cryoglobulin primed mice groups I-IV. These adopted characters can be of help in explanation of the tissue injuries mediated by the tuberculus cryoglobulins. The induced pathologic effects were of dose dependent type.At the same concentration used to prime mice ,different organ tissues showed different type of immune damages.However, as the concentration of cryoglobulin increased the intensity of tissue responses increased.0.2mg./ml., showed only pneumogenic and hepatogenic effects,0.3mg./ml., primed mice have shown; pneumogenic, hepatogenic, lymphogenic ,cardiomyopathic and nephritogenic effects. Incomparision, 0.4mg./ml., primed mice showed; pneumogenic, hepatogenic, lymphogenic, granulomatogenic, cardiomyopathic and nephritogenic effects[12,11]. In lungs, cryoglobulin induce proliferation of epithelial tissue as well as cytokine production . Hyperplasia of; epithelial cells, macrophages and mucosal lymphoid cells is consistent with pulmonary local immune reponses [13,15,6].Perivascular cell cuff and aggregation of inflammatory cells suggest subacute to chronic inflammatory reactions involved in delayed type hyper sensitivity in liver[16].Lymphoid cell hypoplasia may be attributed to regulatory T lymphocytes effect in response to the ac-

tion of cryoglobulin[8].Nephritis and edema in kidneys may be mediated by serum sickness and Arthus reaction[10,9,3].Myocarditis in heart muscle due to inflammatory macrophage activation[9].Thus the immune mediated tissue injuries in mice groups I-III appeared as;type II hypersensitivity in heart muscle ,type III in kidneys and type IV in liver as well as mucosal immune response in lungs. The Conclusions, The human tuberculus cryoglobulin is pathogenic effects for tissue and organs in mice,The pathological effects are immune mediated(cellular immunity),The nature of pathogenic immune mediated was hypersensitivity to some cell body and granuloma formation, Hypersensitivity type II in heart, type III in kidneys and type IV in liver, as well as lungs were showing events of local mucosal immune responses.

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