HISTOPATHOLOGICAL STUDY OF SEPSIS EXPERIMENTALLY INDUCED BY CECAL LIGATION AND PUNCTURE IN RATS

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ABSTRACT

The aim of this study was to investigate the histopathological lesions in liver,kidney and lung sections in rats with sepsis that experimentally induced by cecal ligation and puncture CLP procedure to induce intra-abdominal infection. It was found that CLP models leads to higher mortality rate comparing to sham-operated and un–operated animals. Also there were severe lesions in liver , kidney and lung of rats with sepsis , characterized by severe massive necrosis in hepatic cells , while the histopathological changes in kidney of CLP rats revealed severe glomerulonehritis with infiltration of inflammatory cells , in lung , severe interstitial and bronchial pneumonia , with presence of severe thickening of blood vessels wall with thrombi .In conclusion, the results of this study have demonstrated that CLP induces high mortality rate , anacute systemic septic state with multiple organ dysfunction specially the Liver , kidney and Lungbased on histopathological observations . These features were not observed insham - operated or un-operated rats .
Keyword : Sepsis , Pathological changes , Liver , kidney , lung .

INTRODUCTION

Sepsis is a syndrome involving the systemic host response to an inflammatory or infectious agents.itis a common and frequently fatal condition that occur as a result of severe infection often leading to overwhelming systemicinflammation (1 , 2) , Also The sepsis syndrome is defined by widespread inflammation, host immune dysfunction, dysregulation of the coagulation cascade, and endothelial dysfunction in response to invading pathogens(3).

Cecum ligation and puncture CLP , is currently the most widely used animal model of sepsis (4) . Intra-abdominal infections may be a source for sepsis ,these infections generate a peritoneal inflammatory response to poly-microbial organisms derived from the gastrointestinal tract . Peritonitis may originate from a defect in an abdominal viscus , such as an acute intestinal perforation that progresses to sepsis , resulting in high morbidity andmortality in experimental animals . A shift toward an anti-inflammatory immunosuppressive state has been postulated to occur in the later phase of sepsis (5).

the aim of this study was to investigate the pathological changes of liver , kidney and lung associated with sepsis .

MATERIAL AND METHODS

Animals

White Albino healthy adult rats weighs (200 -250 g) of both sexes were used in this study , kept in plastic cages in a controlled environment ,with free access to foodand water. They obtained from the animal house of the College of Veterinary Medicine , University of Mosul.

Sepsis induction in rats (CLP model)

Polymicrobial sepsis was induced using the CLP model in groupsof rats according to the method described by (6) .Briefly, the animals were anesthetized by a single i.p. injection(0.5 ml) of ketamine (90 mg/kg b.w) and xylazine (10 mg/kg b.w)mixture. A small mid-abdominal incision (2–3 cm) was made, andthe cecum was exposed. A distended portion of the cecum just distalto the ileocecal valve was isolated, filled with fecal content, andtied with a silk suture in a manner not to disrupt bowel continuity.The ligated portion of the cecum was punctured twice with a 20-gauge needle. The cecum was then replaced in its originalposition within the abdomen, and the abdomen was then closedwith a 3 suture in two layers, and the animals were allowed torecover.

In case of sham-operated rats, the cecum was exposed,manipulated and returned to the peritoneal cavity without being ligated andpunctured. After surgery, normal saline(5 ml/100 g b.w) was givensubcutaneously to all rats to prevent dehydration.

Histopathology

Tissue specimens , were collected from the liver, lung and kidney then fixed in 10 % Neutral Buffer formalin solution for 72 h, trimmed to suitable sizes , washed , dehydrated , cleared in xylene , Embedded in paraffine wax, Sectioned at 5-6 µM, stained with hematoxylin and Eosin and examined under a light microscope (7).

Experimental design

We used 18 albino rats (200 - 250 g) , randomly divided into 3 groups, kept in individual cages with food and water standard for rodents . The groups were named as: Group I (n = 6) for the rats not operated (control) ,Group II , Sham – Operated Group (n = 6) and Group III the CLP (n = 6) .

RESULT

Mortality description

Themortality rate was significantly higher in CLP-animals than in Sham- operated animals compared with the control group (Fig. 1) .

24 h after CLP , Mortality was 33% of animals died , and it became 100% after 7 days from CLP – procedure. In Sham- operated group the mortality rate was 10 % during the 24h. then it became zero % after 48h. of the experimental period.
Mortality was Zero in control group during the experimental period .

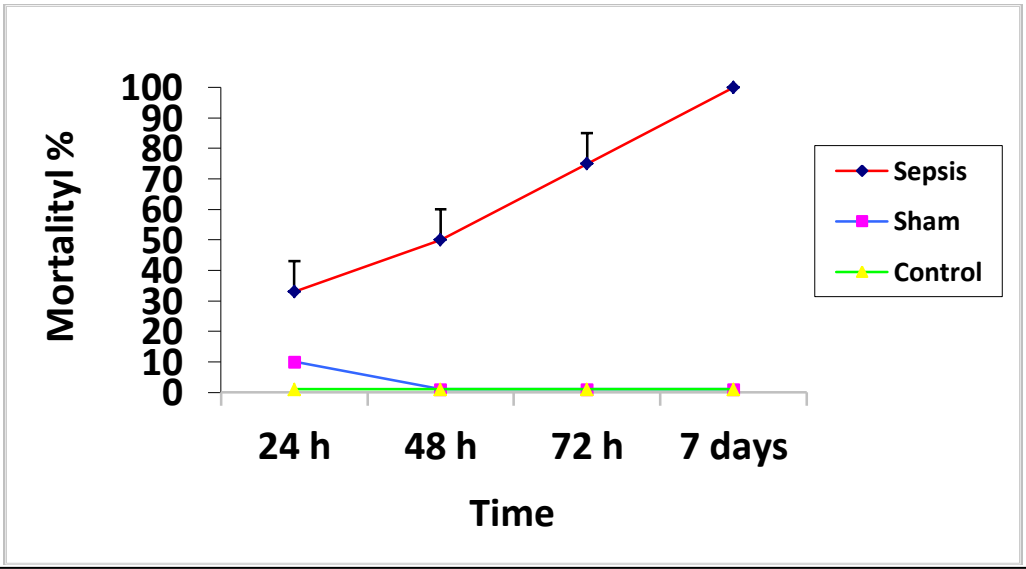

Fig (1) : Mortality rate of rats with CLP , sham – operated and controlgroups.

Histopathological lesions

The microscopic evolutions of liver sections after 24 hours of CLP group revealed fatty change , acute cell swelling and centrilobular necrosis of hepatocytes and apoptotic bodies also seen in the liver with congestion of central vein (Fig. 2) .

While after 3 days of CLP , the histopatgological changes became more severe which characterized by massive necrosis of hepatocytes with dilatation of sinusoids and congestion of central vein , there were infiltration of inflammatory cells in the portal area and around the portal veins (Fig. 3 and 4) , After 7 days of CLP , there were massive necrosis of hepatocytes with focal infiltration of polymorphonuclearinflammatory cells (Fig. 5). While the microscopic changes of sham – operated animals showed vacuolar degeneration and necrosis of hepatocytes (Fig. 6).

The histopathological changes of kidney after 24 hours of CLP , revealed glomerulonephritis which characterized by infiltration of inflammatory cells in the interstitial tissue with hemorrhage in glomeruli associated with acute cell swelling of epithelial renal tubules which lead to stenosis of lumen in many renal tubules (Fig. 7) , while after 3 days to 7 days of CLP , the lesions became more severe which included severe necrosis of mesengial cells of glomeruli with damaged of the basement membrane of the glomeruli with presence of hemorrhage in the interstitial tissue and congestion of blood vessels and necrosis of epithelial renal tubules (Fig. 8). In the sham-operated group mild inflammatory changes were seen (Fig. 9)

In Lung , the histopathological changes after 24 hours of CLP , showed severe inflammatory changes (Interstitial Pneumonia) with pulmonary emphysema (Fig. 10) ,

While after 3-7 days of CLP , the histopathological changes characterized by bronchopneumonia with infiltration of polymorphonuclearinflammatory cells within and around bronchi and severe thickening of blood vessels wall with hemorrhage in the interstitial tissue (Fig. 11) , While other sections showed presence of thrombi in the blood vessles (Fig. 12). In the sham-operated group mild inflammatory changes were seen (Fig. 13).

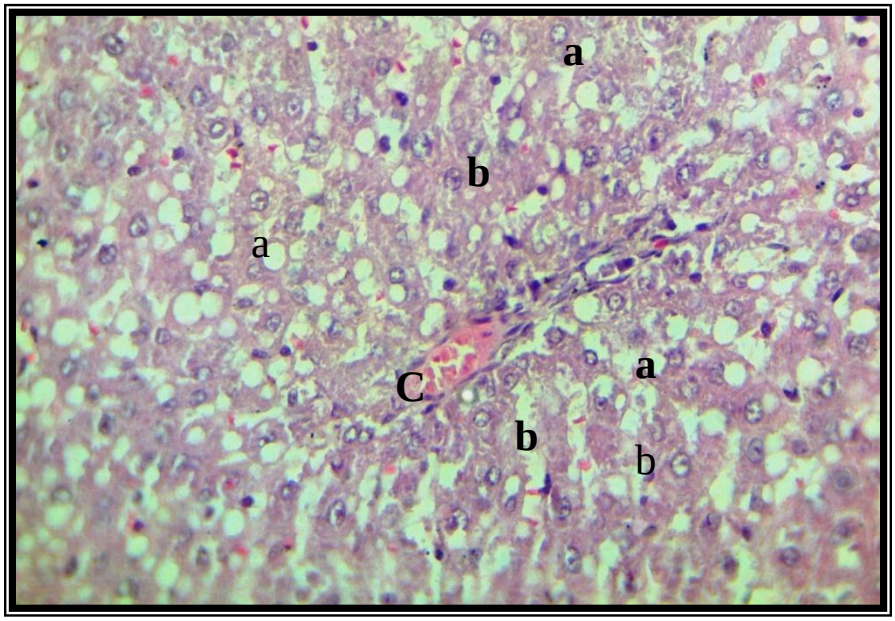

Fig (2) : Rat liver section , 24 hours after CLP , show acute cell swelling (a) , centrilobular necrosis of hepatocytes (b)congestion of central vein (c). H&E X105

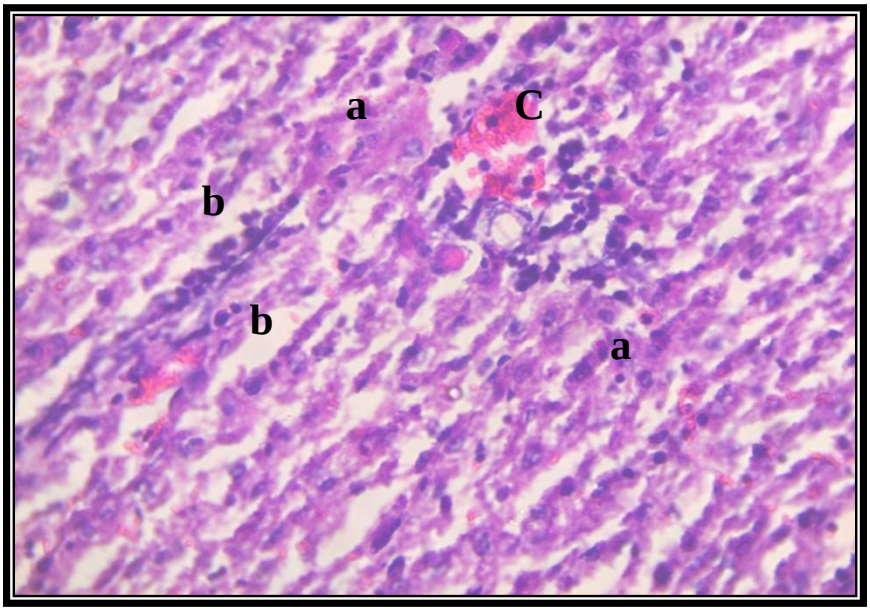

Fig (3) : Rat liver section , 3 days after CLP , show massive necrosis of hepatocytes (a) , dilatation of sinusoids (b) infiltration of inflammatory cells (C) .H &E X105

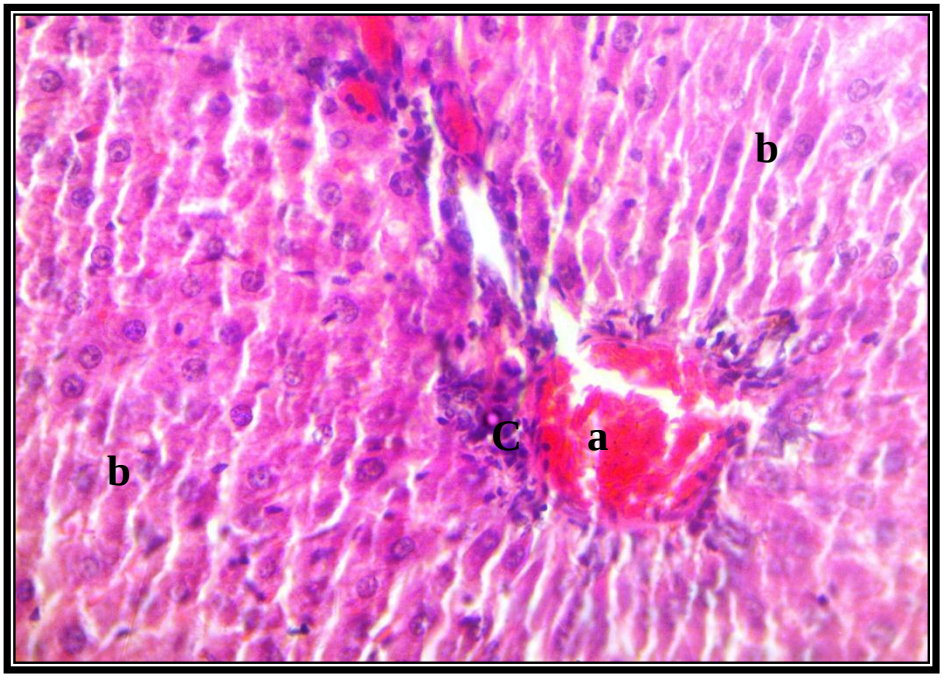

Fig. (4) : Rat liver section 3 days after CLP , show congestion of central vein (a) , severe necrosis of hepatocytes (b) infiltration of inflammatory cells (C) .
H & E. X105

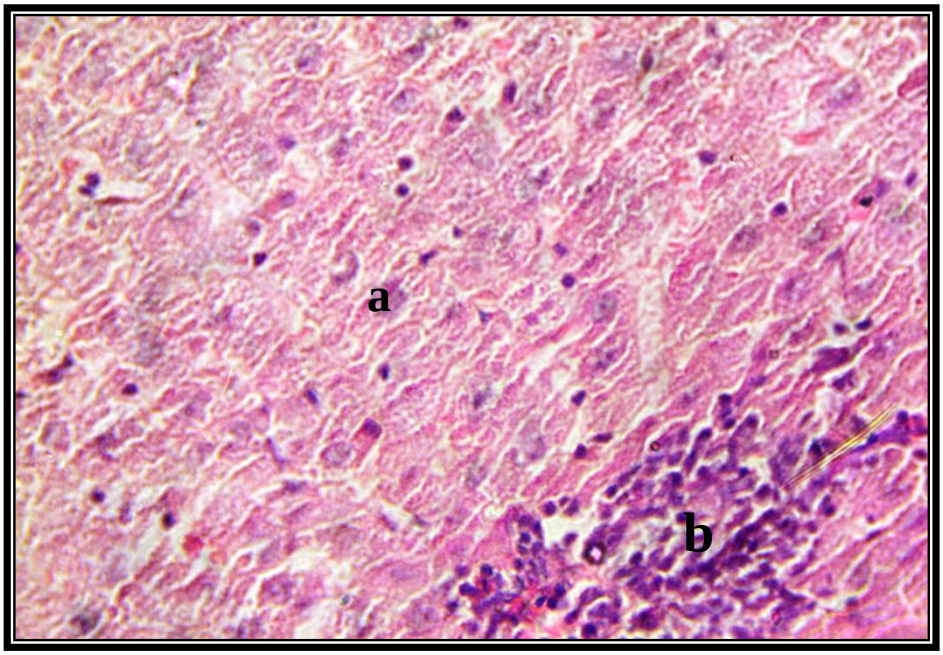

Fig (5) : Rat liver section 7 days after CLP , show massive necrosis of liver parenchyma (a) severe infiltarion of inflammatory cells in portal area(b).
H & E X 105

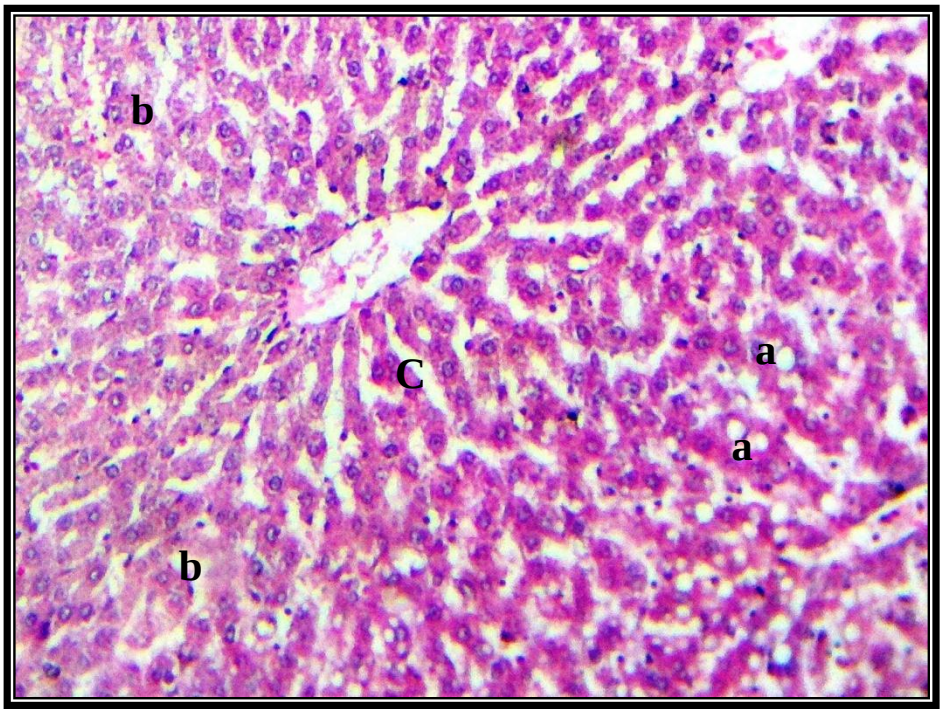

Fig (6) : Rat liver section of sham – operated group , show vacuolar degeneration (a) of hepatocytes ,and necrosis (b) with dilatation of sinusoids (C). H & E X105

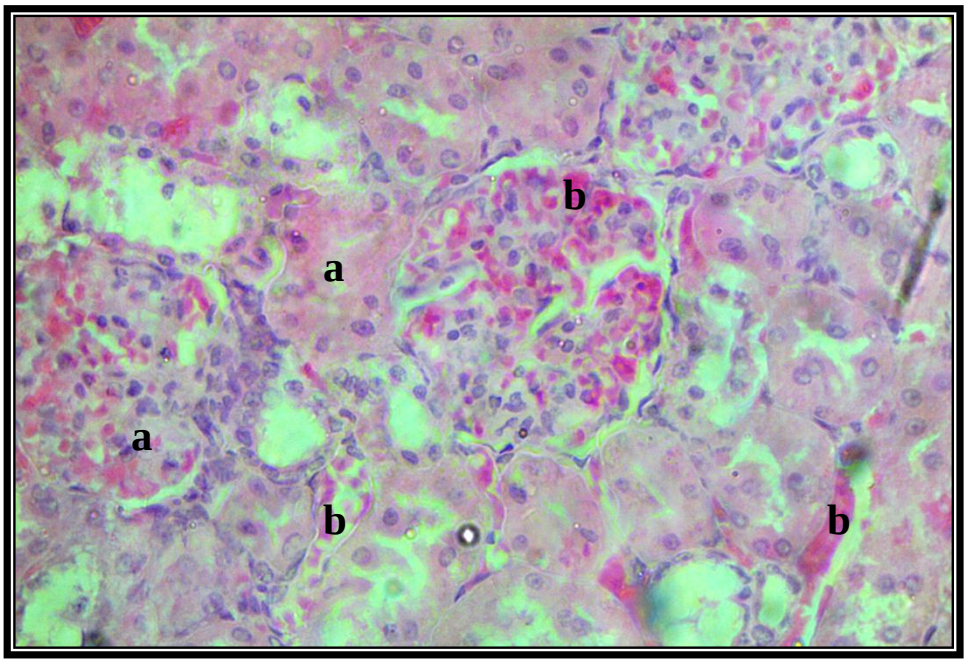

Fig (7) : Rat kidney section 24 hours after CLP , show necrosis in the glomerular and tubular epithelial cells (a) with hemorrhage and congestion in glomeruli and interlobular capillaries (b). H & E X420

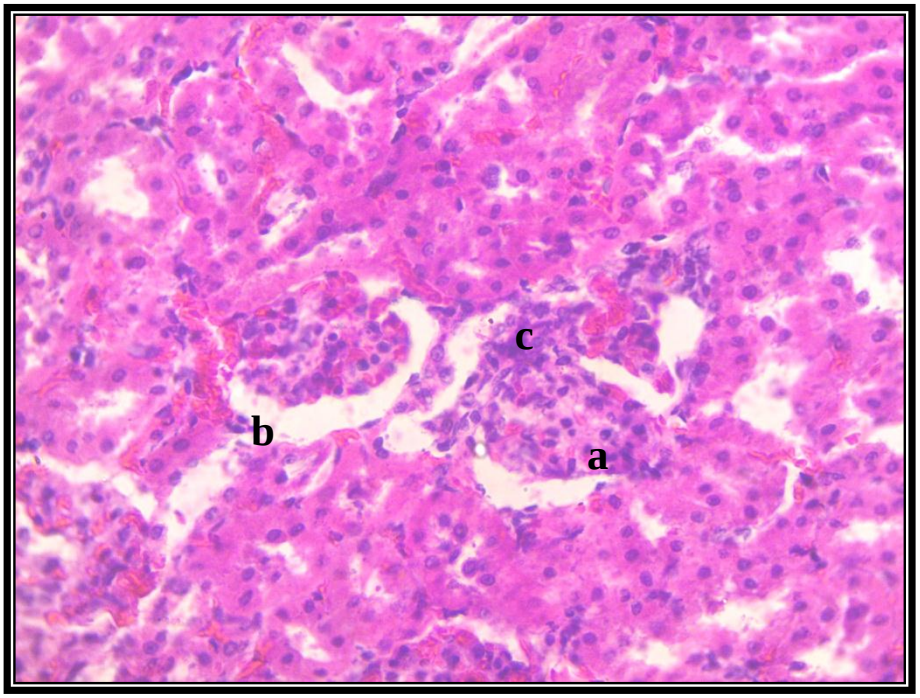

Fig (8) : Rat kidney section 7 days after CLP , show severe necrosis of mesengialcells (a)damaged basement membrane (b) and infiltration of inflammatorycells (c) . H & E stain X 350

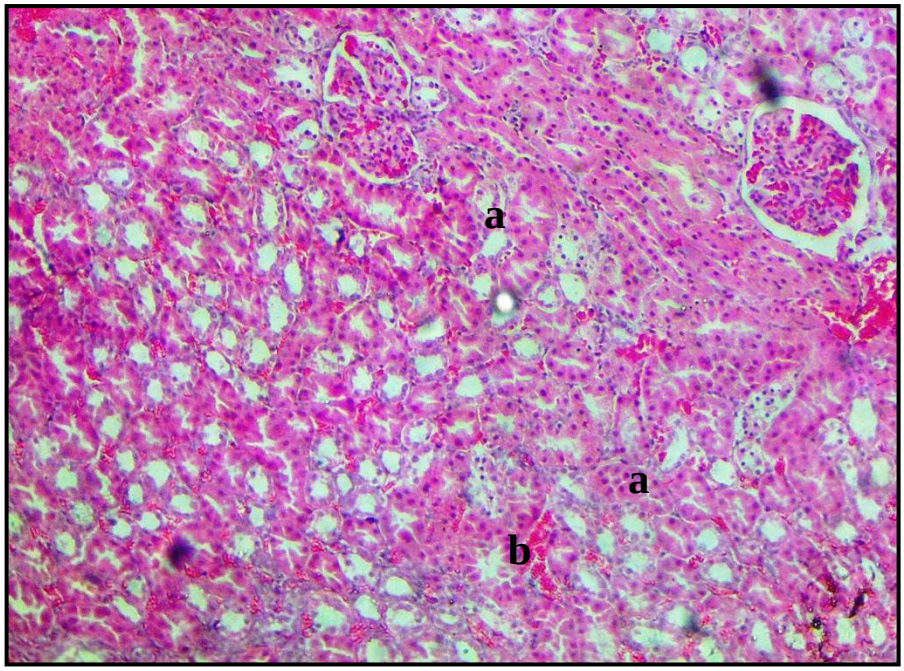

Fig (9) : Rat kidney section of sham – operated group , show cell swelling of epithellium of tubules (a) hemorrhage in renal tissue (b) . H & E stain X 105

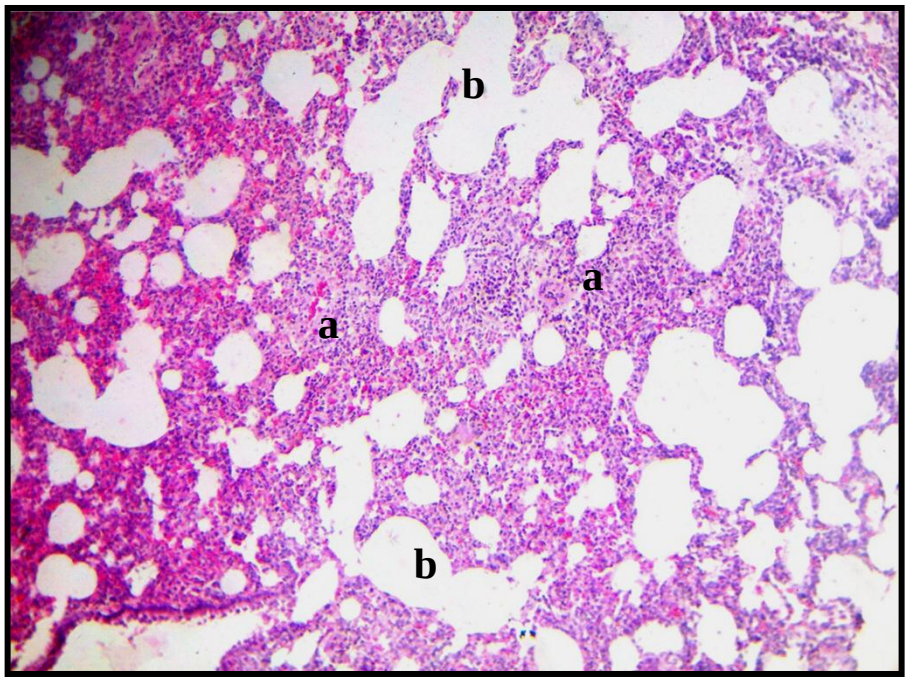

Fig (10) : Rat lung section 24 hours after CLP , show interstitial pnumonia (a) pulmonary emphysema (b) . H & E stain X 105

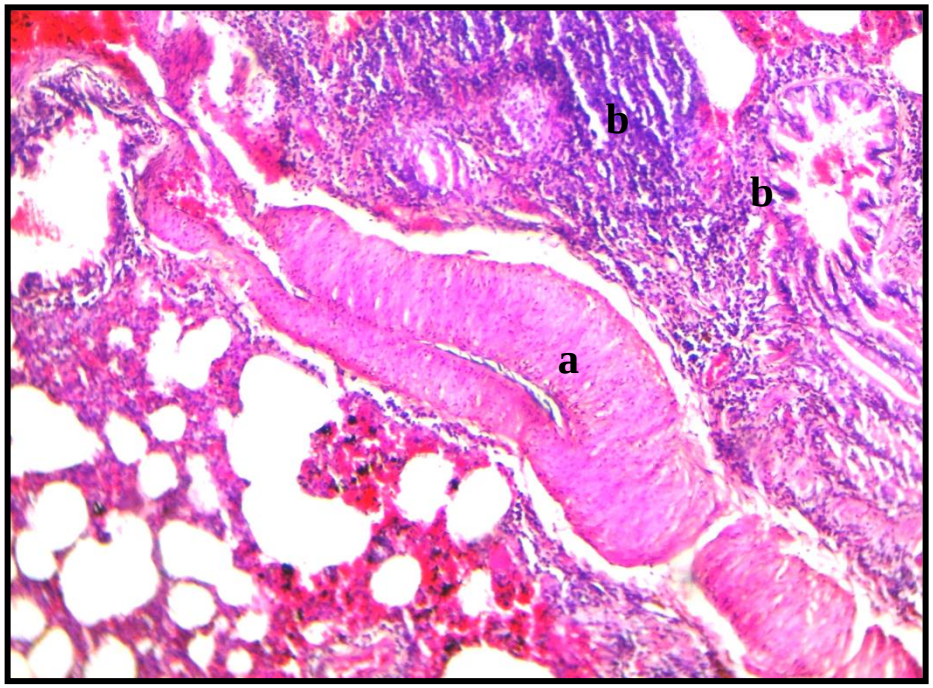

Fig (11) : Rat lung section 7days after CLP , show severe thickening of blood vessels wall (a) severe infiltration of inflammatory cells in the alveoli and bronchiols (b). H & E stain X 105

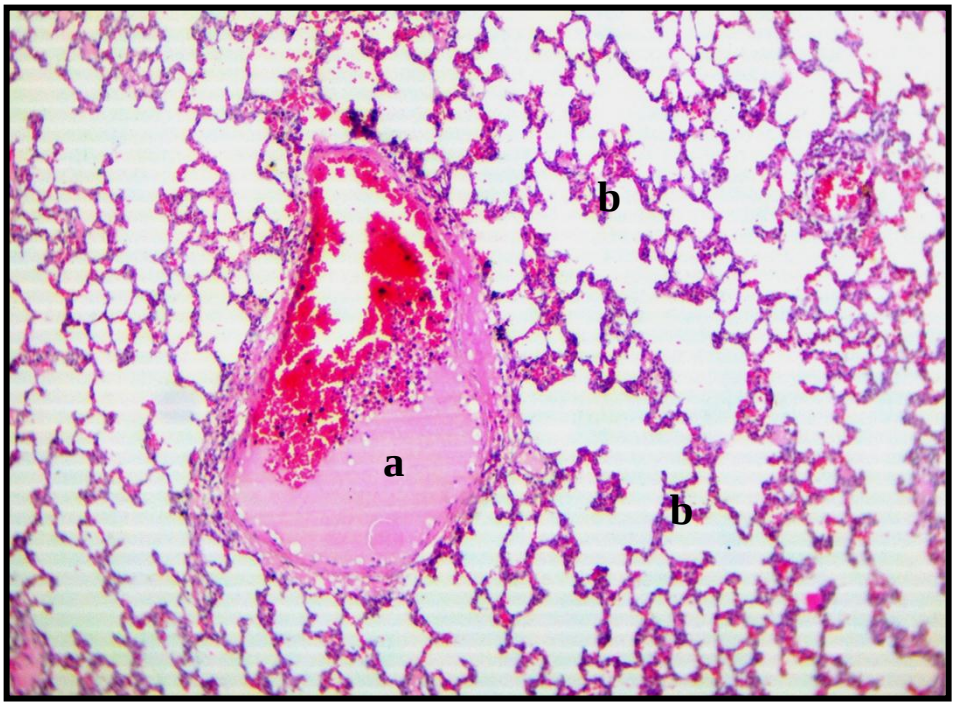

Fig (12) : Rat lung section 7 days after CLP , show thrombus in blood vessel (a) emphysema (b) . H & E stain . X 105

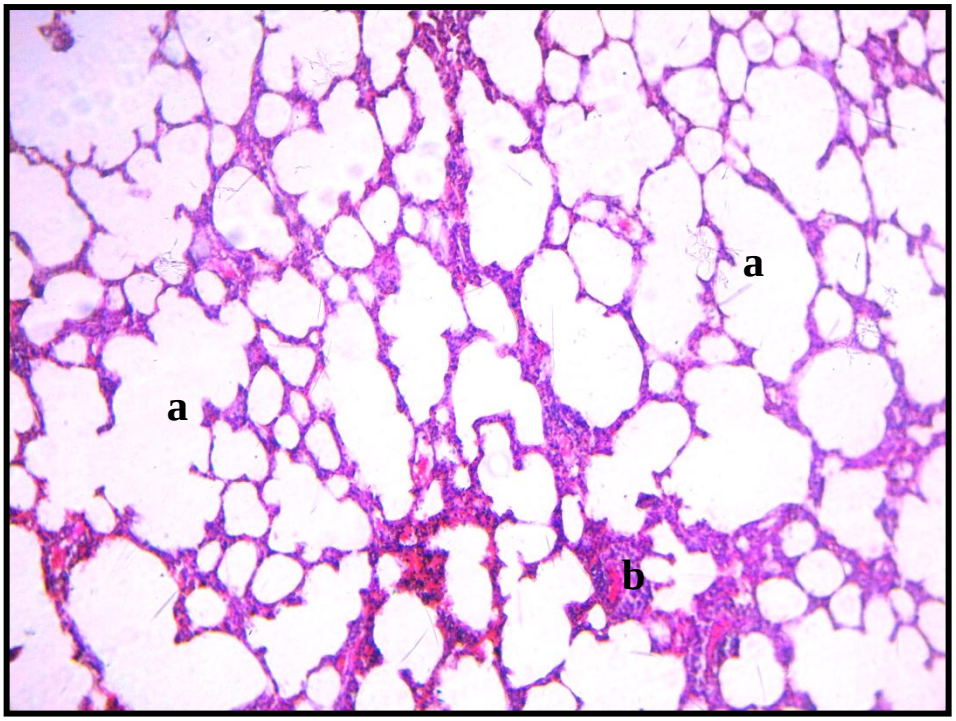

Fig (13) : Rat lung section of sham – operated group , show pulmonary emphysema (a) infiltration of inflammatory cells in the alveolar wall (b) . H & E stain X 105

DISCUSSION

With the present study , we aimed establishing an experimental model of severe sepsis characterized by acute onset and high mortality in the rats . Using the accepted CLP model , as experimental base , we induced gram negative and gram – positive bacterial peritonitis and followed by severe sepsis (8)

In this study we have used surgical operation similar to that described by (9) as a sepsis model by cecal ligation andpuncture (CLP), which led to persistent fecal leakage from the cecum into the abdomen, thus providing apolymicrobial source of infection in rats , which is characterized by an acute onset and high mortality rate (10) .

high mortality rate occurred within 24 hours post CLP and no animal survive after 7 days , comparing with control and sham– operated groups . The acute and high mortality rate result among the experimental animals could be attributed to surgical procedure or manual manipulation of the cecum and intestine , but mostly due to severe and sudden enterance of large numbers of pathogens and its toxins into the peritoneal cavity (11).

The host response towards these invading pathogens is characterized by an over whelming systemic pro–inflammatory response that is primarily mediated by cytokines , which can lead to fatal multiorgan failure and septic shock (12) . This systemic pro–inflammatory response comprises activation of multiple pathways , including cytokines , plasma coagulation and complement cascades , and acute phase proteins release , while the cellular components are in particular leukocytes and vascular endothelium (13).

The histopathological lesions ,that were observed in the Liver , kidney and lung in rats with sepsis are due to sever bacteremia and septicemia that result from rapid transfer of septic pathogens and its toxins from the peritoneal cavity into the systemic circulation . Gram negative aerobes and Facultative anaerobes such as *E. coli* which consider the most common pathogens agents that identified in circulation in man and laboratory animals (14) .

The hepatic histopathology of rats subjected to sepsis showed areas of necrosis , and this is may be due to the effect of bacterial toxins because CLP results insepticaemia of faecal origin as well as due to to overt generation ofcytokines, eicosanoids, and reactive oxygen species , which leads to endothelial cell damage, formation of chemotatic factors,recrutment of neutrophils, lipid peroxidation, and oxidation,DNA damage, release of tumor necrosis factor(TNF)-α, and interleukin (IL). (15)

During sepsis the most common injury to the kidney is acute tubular necrosis, caused by renal hypoperfusion, partly responsible for the acute renal failure , which is characterized by destruction of Glomerular and tubular epithelial cells. Among the structural damage, cellular swelling, detachment of cells and lethal injury (necrosis) are found and these results are agree with (16). The necrotic cell compromises cellular membranes that allow leakage of injurious, proteolytic enzymes , These enzymes leak into the cytoplasm fromintracellular organelles, such as lysosomes, or into the surrounding tissue. Alsofollowing transmigration and activation, infiltrating neutrophils produce abundant oxygenradicals ***via*** oxidative bursts. other sources of oxygenradical species include activated macrophages , these oxygen radicals areresponsible for cellular

lipid peroxidation, protein oxidation, and mitochondrial impairment function,which cause further damage to tissues and can inducecell death.(17)

The lung is one of the most common organs affected in sepsis , and cellular infiltration, together withthe release of proinflammatory mediators, leads to the developmentof acute lung injury , characterized by edema, hemorrhage , destruction of alveolar wall with severe infiltration of inflammatory cells and these result agree with (18). It is nowwidely accepted that the formation of inflammatorymediators plays an important role in the pathophysiologyof inflammation in acute lung injury (19) .

Another recent area of interest in the pathogenesis of lung injury has been the role of reactive oxygen species . Apart from direct cytotoxic effects, reactive oxygen species have important effects on the inflammatory response mediated via changes in oxidant/antioxidant balance.(15) .

In conclusion, the results of this study have demonstrated that CLP induces high mortality rate , anacute systemic septic state with multiple organ dysfunction specially the Liver , kidney and Lungbased on histopathological observations, these features were not observed in sham - operated or un-operated rats .

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دراسة مرضية نسجية للانتان المحدث تجريبياً بواسطة ربط الاعور وثقبه في الجرذان

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الخلاصة

الهدف من هذه الدراسة هو الكشف عن الافات المرضية النسجية لكل من الكبد ، الكلية والرئة الناجمة عن الانتان في الجرذان والمحدث تجريبياً بطريقة ربط الاعور وثقبه وذلك لاحداث الخمج داخل البطن ، حيث أظهرت عملية ربط الاعور وثقبه نسبة نفوق عالية في الجرذان مقارنة مع حيوانات السيطرة ومجموعة الحيوانات التي عوملت جراحياً بدون ربط الاعور وثقبه .

أظهرت التغيرات المرضية النسجية لكل من الكبد ، الكلية والرئة لمجموعة الجرذان المعاملة بربط الاعور وثقبه ، افات شديدة تمثلت بالنخر الشديد الخلايا الكبدية ، بينما أظهرت التغيرات المرضية النسجية لمقاطع الكلية التهاب الكلية الكبيبي الشديد مع ارتشاح للخلايا الالتهابية ، أما في الرئة ، ذات الرئة الخلالي الشديد وذات الرئة القصيبي ، فضلاً عن تثخن شديد في جدران الاوعية الدموية مع وجود الخثرة هي اهم الافات المميزة .