Research article

Evaluation of liver and renal function tests together with histopathological alterations in rabbits infected with a virulent strain of *Proteus vulgaris*

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ABSTRACT

Introduction and Aim: Gram-negative bacterium *Proteus vulgaris* is found in animal and human guts. This bacterium can cause renal and vesical calculi, septicemia, and wound infection-related pyrexia. Antibiotics may not cure respiratory infections. This study infected rabbits with a virulent strain of P. vulgaris from bovine mastitis to examine metabolic and histological changes in various organs.

Materials and Methods: This study was carried out on twenty domestic rabbits of both sexes which were divided into two groups. The first group received 10^{10} CFU\ml\ of virulent *Proteus vulgaris* subcutaneously. While the second group (control) received subcutaneously 1 cc of phosphate buffered saline (pH = 7.2). On 3,7, 14 days of infection, the animals were checked for their biochemical parameters (AST, ALT, ALP, blood urea and creatinine). Histological alterations in various organs were also studied.

Results: The infected group showed substantial increases ($P \leq 0.01$) in liver enzymes (AST, ALT, and ALP) at 3, 7, and 14 days compared to the control group. In contrast, the infected group showed a substantial rise ($P < 0.01$) in blood urea and creatinine levels at 3, 7, and 14 days compared to pre-infection levels and the control group. Histological analysis of lung tissue showed dilated alveolar gaps with hemorrhagic and fibrinous exudates and many alveolar macrophages. These findings included pulmonary vasodilation and congestion. Intramuscular edema, cellular infiltration, intramuscular hemorrhage, and congestion was present in heart tissue with little muscle fiber separation. The intestines had significant patch proliferation and mononuclear cell infiltration. Blood vascular congestion and mononuclear cell infiltration were also seen in the liver and kidney.

Conclusion: Experimental infection with pathogenic bacterium *Proteus vulgaris* raised rabbits' blood urea, creatinine, and liver enzymes AST, ALT, and alkaline phosphatase. Cardiovascular, renal, hepatic, intestinal, and pulmonary tissues showed significant postmortem changes.

Keywords: *Proteus vulgaris;* liver and renal function test; rabbits; histopathology.

INTRODUCTION

Proteus bacteria are members of the genus *Proteus*, belonging to the *Enterobacteriaceae* family (1). Proteus bacteria are Gram-negative facultative human and animal pathogens that primarily infect elderly patients or debilitated animals because of their compromised immune systems. It causes urinary tract infections and these infections are frequently followed by consequences such as pyelonephritis and the development of urine stones (2). Additionally, they are also implicated in kidney and bladder stone formation, fever, septicemia, as well as respiratory infections (3). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), which are increased in both myopathy and hepatitis but largely specific to the liver and muscle, respectively, in ALT, are the serum enzymes most commonly used to identify myopathy and hepatitis (4). The complex vascular and biliary system in the liver was one of the damaged organs due to the pathological alterations primarily brought on by infection by bacterial species antigens that were utilized to immunize the experimental rabbits. A sign of hepatic injury in several animals, the enzymes ALT and AST are mostly found in the cytoplasm of hepatocytes (5). The membrane-bound enzyme ALP, which is present in a range of tissues, is recognized to serve a purpose in other tissues (6). Serum ALT is found in high concentration in liver and to a lesser amount in skeletal muscle, kidney, and heart (7). The enzyme ALP hydrolyzes phosphates at an alkaline pH. Several isoenzymes' activity is included in the activity assessed by common methods. They can be present in the placenta, bone, liver, kidney, intestinal wall, and lactating mammary gland. Osteoblasts in bone contain the enzyme, which is presumably crucial for typical bone production. Alkaline phosphatase levels in adults are typically generated primarily from the liver. Due to the placenta's creation of a heat-stable alkaline phosphatase, pregnancy raises normal ranges **(**8). *Proteus vulgaris* has a variety of virulence factors, which may have a role in adhesion to the uroepithelium and other severe side effects such the development of kidney and bladder stones (9). *Proteus* spp. cause 1-2% of urinary tract infections in healthy women and 5% of hospital-acquired infections; they also affect more men than women in newborns and are more prevalent in people between the ages of 20 and 50 (4). In view of the possible damage that this bacterium might inflict in

the form of infections, a number of investigations and publications have started looking into alternative methods to reduce the detrimental consequences of different microbial activity (10-12). The objective of this study was to identify liver function enzymes, specifically Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as conduct renal function tests including blood urea and creatinine. Additionally, the investigation aimed to examine postmortem changes in various organs, such as the heart, kidney, liver, intestine, and lungs, following experimental infection with virulent *P. vulgaris* bacteria.

MATERIALS AND METHODS

Bacterial isolation

The bacteria *Proteus vulgaris* were found in an instance of clinical mastitis in a cow. Following antisepsis, milk samples (n=120) from clinical cases were obtained and cultured in Brain Heart Infusion broth. The recovered bacteria were streaked onto MacConkey agar and blood agar plates after being cultivated overnight in BHIB at 37°C for 48 hours. The plates with the inoculum were aerobically incubated. Colony morphology, Gram staining, carbohydrate fermentation (sucrose, mannitol, lactose, and glucose), and biochemical (indole, citrate, TSIA, catalase, and urease) assays were used to identify every single colony. The Vitek-2 system was used to confirm the diagnosis, (13). The isolation process was made by the clinical pathology lab at the University of Diyala, College of Veterinary Medicine, Diyala, Iraq.

Animals

Twenty rabbits, each free of a specific disease and weighing 1.5–2 kg, were housed in appropriate cages in an appropriate air-conditioned housing unit and left for a few weeks to adjust while receiving a healthy diet with unlimited access to food and water. The Institutional Animal Care and the Ethics Committee both gave their approval to the procedures. The animals were split into two groups of ten each at random. The first group received 10^{10}CFU\ml subcutaneous injection of virulent *Proteus vulgaris*. While the second group received 1 cc of phosphate buffered saline (pH = 7.2) S/C as the control.

Blood sample collection

Blood (5 ml) was obtained aseptically from each animal at 3,7, and 14 days via jugular vein puncture were transferred to sterile test tubes and allowed to coagulate in a refrigerator for 24 hours to obtain serum. The serum obtained was stored at -20 C° until further use.

Biochemical assays

Serum was subjected to standard liver tests (Alanine Aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)) and renal function tests (Blood urea and serum creatinine). The Enzyme-linked Immunosorbent Assay kits were used to measure the liver enzymes ALT (Cloud-clone. SEA207Mu, Houston-USA), AST (Eiaab. E1214h, USA) and ALP (KT-52742 1-5, Seattle, USA). The kidney function tests (Blood urea and serum creatinine) were performed using kits (Bio system, Spain) according to manufacturer's instructions.

Histopathological examination

After the experimental period the animals were sacrificed and tissues (1 cm3 diameter) were collected from various organs of infected and control rabbits. The Heart, lung, kidney, liver, and intestinal tissues were collected over the course of five successful days, beginning at 24 hours after infection and continuing until 168 hours after infection. The tissue samples collected were dehydrated by processing with a gradient of alcohol with a concentration ranging from 70% to 100% in intervals of 10% for two hours after being fixed with buffered formalin of 10% for 72 hours. Following this the tissues were treated with xylol, and two stages of semi-liquid paraffin wax embedding at 58°C. Using a microtome, 5 µm thick sections of the finished processed tissue were cut from paraffin blocks. Hematoxylin and eosin (H&E) staining of cut tissues on glass slides was done to look for histopathological changes in contrast to control tissues under a light microscope (14).

RESULTS

The findings of the ALT enzyme after 3, 7, and 14 days showed a substantial increase ($P \leq 0.01$) in the infected group, with values of (71.31± 1.5), (80.33± 1.4), and (75.22 ± 1.7) respectively, in comparison to the control group's values of (45.23± 1.7), (44.36± 1.4), and (45.22± 1.6) respectively (Table 1). The infected group's levels of the AST enzyme increased significantly ($P \leq 0.01$) after 3, 7, and 14 days, with values of (90.21 ±1.1), (94.23±1.2), and (85.21± 2.2), compared to the control group's values of (49.13 ± 2.1), (50.34 ± 2.4), and (50.12 ± 1.1), respectively (Table 1).

Table 1: The levels of ALT, AST and ALP enzymes following infection with virulent *Proteus vulgaris* in rabbits

Liver enzyme (µg/L)	Groups	Days		
		3 days	7 days	14 days
Alanine Aminotransferase (ALT)	Infected group	71. 31±1.5^{Aa}	80.33± 1.4^{Ab}	75.22± 1.7^{Aab}
	Control	45.23 ± 1.7^{B}	44.36 ±1.4^{B}	45.22 ± 1.6^{B}
Aspartate aminotransferase (AST)	Infected group	90. 21± 1.1^{A}	94.23 ± 1.2^{A}	85.21 ± 2.2^{A}
	Control	49.13 ± 2.1^{B}	50.34 ± 2.4^{B}	50.12 ± 1.1^{B}

Alkaline phosphatase (ALP)	Infected group	63. 31± 1.1^{A}	74.13 ± 1.2^{A}	65.22 ± 2.2^{A}
	Control	40.11 ± 2.1^{B}	41.31 ± 1.3^{B}	41.22 ± 1.7^{B}

Capital letters = differences inside the same group, small letters = differences between the different groups, same letters = no significant differences (P ≥ 0.01), the different letters = significant differences (P ≤0.01**).** The various vertical letters denote the groups' significant (P <0.01) difference.

Table 2: Rabbit blood urea and serum creatinine levels after infection with virulent *Proteus vulgaris*

Enzyme (µg/L)	**Groups**	**Days**			
		0 Day	**3 days**	**7 days**	**14 days**
Blood urea	Infected group	31. 21± 1.4^{Aa}	80.22± 1.1^{Ab}	87.31± 2.2^{Ab}	90.31± 1.1 ^{Ab}
	Control	30.10 ± 2.2^{A}	31.31 ± 1.2^{B}	31.31 ± 2.1^{B}	30.24 ± 2.2^{B}
Serum creatinine	Infected group	1.32± 1.3^{Aa}	5.22± 2.1^{Ab}	7.23± 1.1^{Ab}	3.9± 1.3 ^{Ab}
	Control	1.10 ± 2.3^{A}	1.21 ± 1.3^{B}	1.12 ± 2.1^{B}	1.14 ± 1.2^{B}

Capital letters = differences inside the same group, small letters = differences between the different groups, The same letters = no significant differences (P ≥ 0.01), The different letters = significant differences (P ≤0.01**).**

After 3, 7, and 14 days, the infected group's ALP enzyme levels significantly (P≤ 0.01) increased, reaching values of (63. 31± 1.1), (74.13± 1.2), and (65.22± 2.2), respectively, as opposed to the control group's values of (40.11± 2.1), (41.31± 1.3), and (41.22 ±1.7), respectively (Table 1).

Kidney function tests

The blood urea results for the control group (30.10 ± 2.2) and infected (31.21±1.4) groups were within the normal range before infection (zero time). When compared to a control group or zero time, the infected group reported a significant increase (P ≤ 0.01) in 3, 7, and 14 day (80.22± 1.1), (87.31± 2.2), and (90.31± 1.1), respectively (Table 2).

In case of serum creatinine, the results before infection (0 day) showed normal values for the infected and control group (1.32± 1.3), (1.10 ± 2.3) respectively. After infection the infected group showed significant increase (P ≤ 0.01) in 3,7 and 14 day (5.22 ± 2.1), (7.23 ± 1.1) and (3.9 ± 1.3) respectively, when compared with the result before the infection or with control at the same time (Table 2).

Histopathological examination

The pathological changes in rabbit tissues caused by exposure to the virulent *Proteus vulgaris* have been examined under a microscope. During extensive inspection, changes were discovered at various organs, including:

Lung

Microscopic section of lung tissue from rabbits inoculated with *Proteus vulgaris* showed the alveolar space distended with hemorrhagic – fibrinous exudates mixed with number of alveolar macrophages accompanied with pulmonary vasodilation and congestion Fig. 1 and Fig 3 also evidence of active hyperemia and edema of alveolar tissue with macrophage and neutrophil infiltration in alveolar space Fig. 2 and Fig 4. Some of the alveolar spaces are filled with liquefied materials with a number of hemosiderin laden macrophages (Fig. 5).

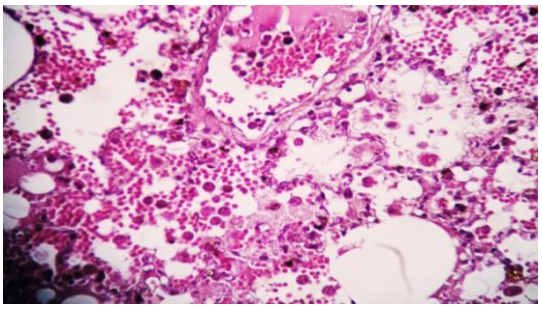

Fig. 1: infected lung tissue showed the alveolar spaces filled with RBCs, neutrophil, with edematous fluid and blood vessels congestion. (H&E X40).

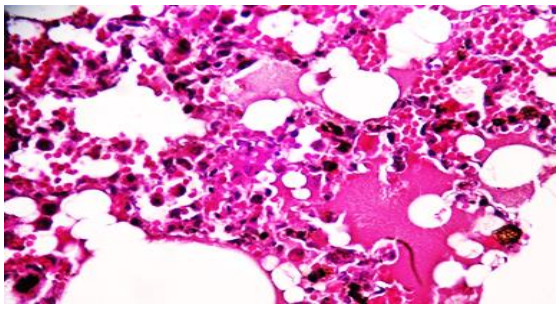

Fig. 2: Infected lung tissue from rabbits showed active hyperemia and edema of pulmonary parenchyma with neutrophils and alveolar macrophage infiltration (H&E X40).

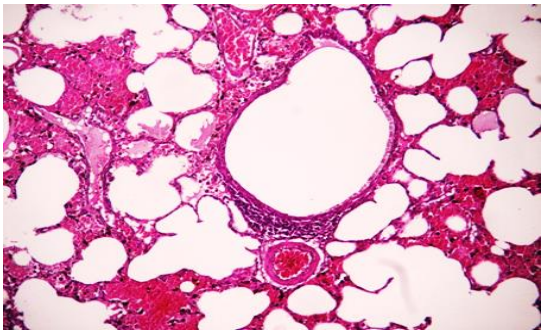

Fig. 3: infected lung tissue showed bronchiectasis (H&E X40).

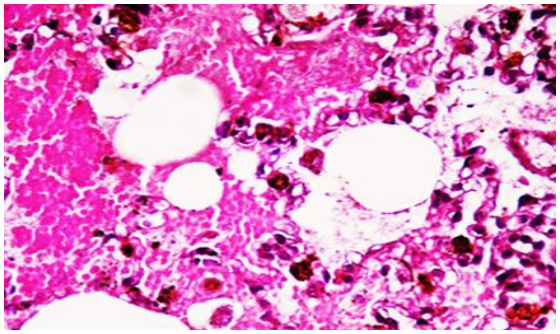

Fig. 4: infected lung tissue showed Liquefied inflammatory exudate with hemosiderin Laden Macrophage. (H&E X40).

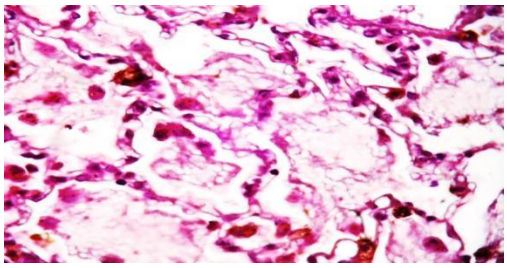

Fig. 5: infected lung tissue showed most alveoli filled with dense fibrinous exudate with scattered neutrophils. (H&E X40).

Heart

The Microscopical section of heart tissue from rabbits inoculated with proteus vulgaris in which mild separation of muscle fibers with intramuscular edema including few cellular infiltration and intramuscular hemorrhage and congestion (Figs. 6 and 7). Also the cardiac manifestation showed focal interstitial hemorrhage with evidence of muscle fiber atrophy, mild hyalinization of cardiac muscle fibers with mild interstitial mononuclear cells infiltration vascular congestion and dilation between muscles bundles (Figs. 8 and 9).

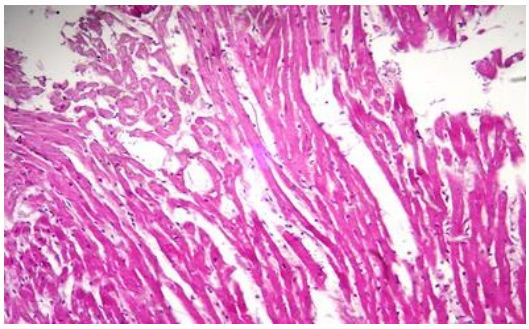

Fig. 6: infected heart tissue in which cardiac muscle Separation with mild interstitial inflammatory edema and few cellular infiltrates (H&E X10).

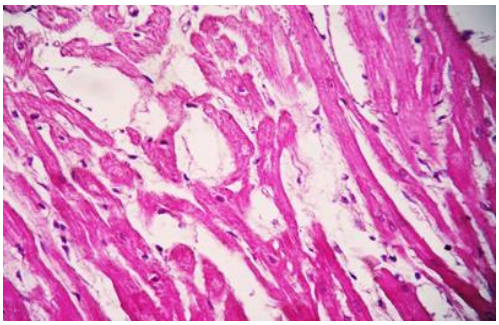

Fig. 7: infected heart tissue in cardiac muscle fragmentation with intramuscular Edema containing few inflammatory cells. (H&E X40)

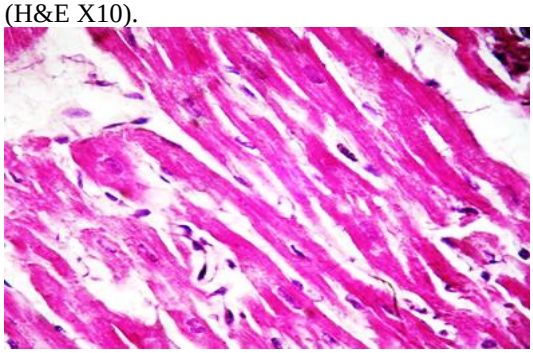

Fig. 8: Infected heart tissue showed mild hyalinization of cardiac muscle fiber with mild interstitial mononuclear cells infiltration (H&E X40).

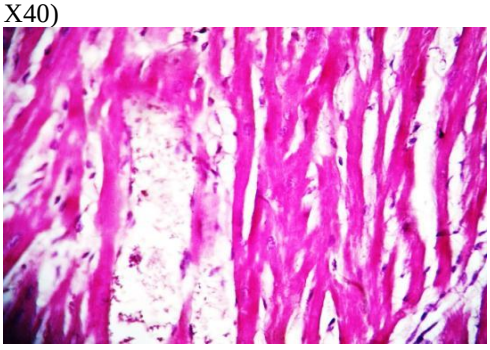

Fig. 9: Infected heart tissue showed focal interstitial hemorrhage with atrophy of adjacent muscle fibers (H&E X40).

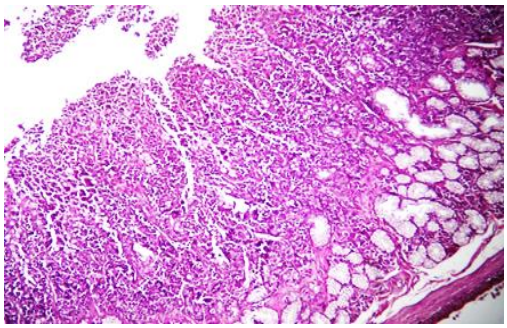

Fig. 10: Infected intestine tissue showed diffuse and inflammatory cell infiltration in both Mucosal and submucosal layer (H&E X10).

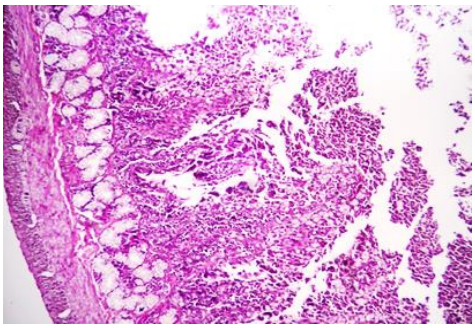

Fig. 11: Infected intestine tissue showed diffuse marked cellular infiltration in mucosal and submucosal tissue. (H&E X40)

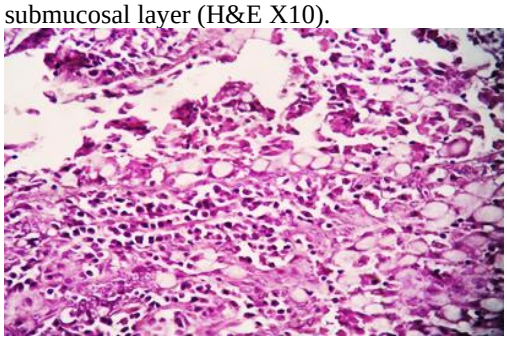

Fig.12: Infected intestine tissue in which goblet cell with necrotic lesion of adjacent mucosal tissue. (H&E X40).

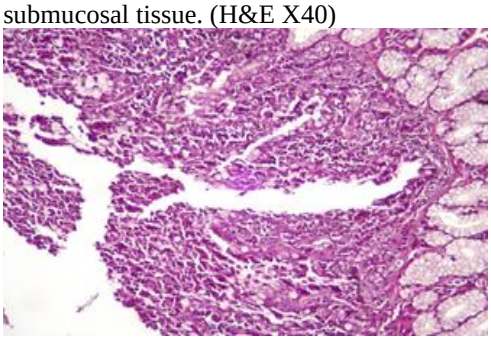

Fig.13: Infected intestine tissue showing goblet cell hyperplasia with necrotic and ulceration lesion of deep mucosal tissue. (H&E X40).

Intestine

The Microscopical section of intestine tissue from rabbits inoculated with proteus vulgaris in which diffuse inflammatory cell infiltration is associated with moderate necrotic and ulcerative lesion of mucosal tissue (Figs. 12 and 13). Also, goblet cell hyperplasia was observed in other sections accompanied with moderate mucosal mononuclear cell infiltration and necrotic findings of adjacent mucosa (Figs.10 and 11).

Liver

The hepatic tissue sections showed focal neutrophilic accumulation around small bile ductuli surrounded with scattered apoptotic hepatocyte and prominence of Kupffer cells (Fig.14), also congestion of hepatic sinusoids (Fig. 15), and portal vein with perivenular leucocytic infiltration (Fig. 16).

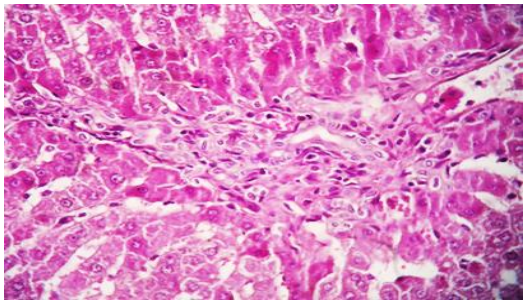

Fig.14: Infected liver tissue in which focal neutrophil accumulation around small bile ductile with scattered apoptotic hepatocytes (H&E X40).

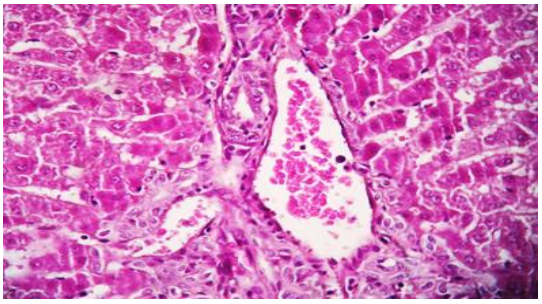

Fig. 15: Infected liver tissue showed focal hepatic cell necrosis and portal vein congestion with peri-Venular Leucocyte infiltration (H&E X40).

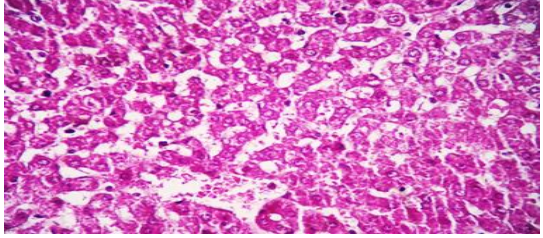

Fig. 16: Infected liver tissue showed hepatic sinusoids congestion and dilatation with kupffer cell prominence (H&E X10)

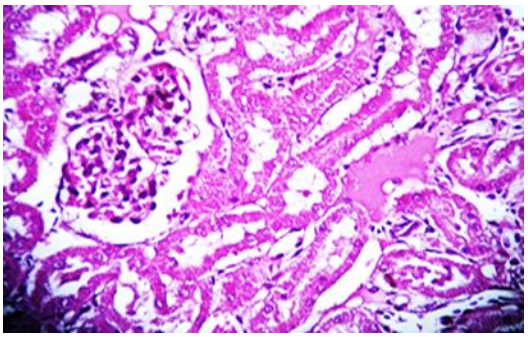

Fig.17: Infected kidney tissue showing presence of edematous substance between degenerated tubules with few interstitial leukocytic infiltration (H&E X40).

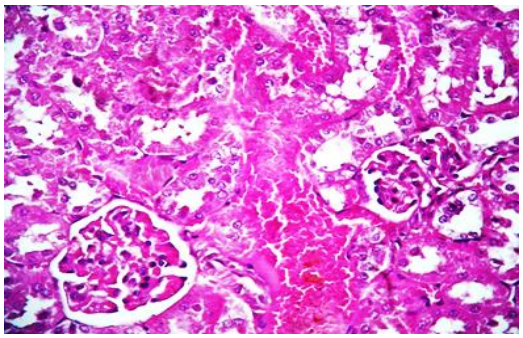

Fig.18: Infected kidney tissue in which renal vessel congestion and dilation with mild Congestion of glomerular tuft (H&E X40).

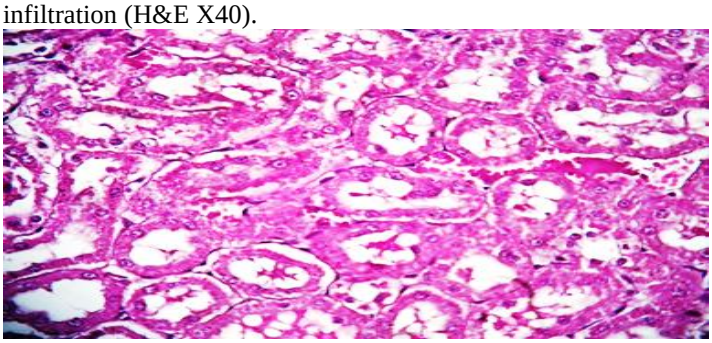

Fig.19: Infected kidney tissue reported Marked cystic tubular dilation with mild epithelial Desquamation of some tubules. (H&E X40).

Kidney

The microscopical section of kidney tissue from rabbits inoculated with *Proteus vulgaris* showed the presence of edematous degenerated tubules accompanied with few leukocytic infiltrates (Fig.17). Blood vessel congestion was noticed in cortical renal area with mild change of adjacent glomeruli Fig (18). Varying degrees of tubular dilation with cystic appearance with vacuolation of tubular epithelial lining was seen (Fig 19).

DISCUSSION

Proteus vulgaris is a rod-shaped Gram-negative bacteria infecting humans and animals. Infection by *P. vulgaris* can lead to severe liver and kidney damage (2). Destruction of hepatic cells can result in elevation of liver enzymes such as arginase, glutamic dehydrogenase (AGD), iditol dehydrogenase (ID), lactic dehydrogenase, Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and leucine aminopeptidase (LAP) (15). The results of our investigation showed that infection with the *P. vulgaris* bacteria increases the levels of the liver enzymes AST, ALT, and ALP in blood. This is consistent with a study with *Salmonella paratyphi* an infection, reported wherein a significant increase in the serum ALP, AST and ALT levels was reported (16). Similarly, the liver enzymes (ALP, ALT, AST, and GGT) were reported to be considerably elevated in rabbits inoculated with sonicated antigens of *Klebsiella pneumoniae* (17). Our research also showed an increase in renal function tests such as (B. urea) and (S. creatinine), with the infected group showing a significant increase in 3, 7, and 14 days. After the bacterial infection was diagnosed, renal impairment was characterized as an abnormal peak serum creatinine level (>1.5 mg/dL) and a serum creatinine level increase of more than 50% over the baseline value (18). Other studies that looked at renal dysfunction following bacterial infection found that blood urea nitrogen, serum sodium concentration prior to peritonitis, and band neutrophils count at diagnosis were all independent predictors of the development of renal dysfunction (19).

Results in this study for microscopic analysis of lung tissue from rabbits infected with *Proteus vulgaris* showed the alveolar space distended with hemorrhagic – fibrinous exudates mixed with number of alveolar macrophages accompanied with pulmonary vasodilation and congestion, which is in agreement with an earlier study (20), who reported blood capillary congestion and polymorphonuclear cell infiltration 3 days after the test. At 20 days after the challenge, the same lesion returned along with thickening of the interalveolar septa brought on by congestion and MNC infiltration. In addition to hyperemia (severe capillary congestion), polymorphonuclear leukocyte infiltrates, edema, alveolar hemorrhage, bronchiolar hyperplasia, and purulent exudates inside the bronchus and bronchioles, acute pneumonia is characterized by these symptoms. A bacterial pathogen must manage to get past the bronchus's first mechanical defense, mucociliary clearance, in the early stages of a respiratory illness. Histopathological changes in vital organs of rabbits due to *Corynebacterium pseudotuberculosis* has also been reported (21) where the infected lung tissues revealed prominence of goblet

cells with moderate mononuclear cells in lamina propria, primarily consisting of plasma cell and macrophages, at the same time as tissues revealed villus lining with clear columnar enteric cyst associated with moderate hyperplasia of submucosal gland without evidence of inflammatory response. The liver itself may become infected by bacteria, toxins in the blood, ischemia, or a combination of these factors. Through the portal vein or hepatic artery, germs can infect the liver. The most prevalent pathological finding is canalicular cholestasis, which is most severe in the perivenular regions. This condition is accompanied by varying degrees of Kupffer-cell activation, lipid alteration, and portal inflammation, but often little to no hepatocellular necrosis (22, 23). On the other hand, the results for kidney tissue alterations in rabbits inoculated with *P. vulgaris* seen in this study was consistent with those recorded during a previous study involving the effect of *Klebsiella pneumoniae* antigens on the immune response in rabbits (24). In another study, mononuclear cell aggregation together with sloughed epithelial cells, severe swelling and degeneration of epithelial cells of tubules was reported in rabbit kidney following injection with *Klebsiella* strain (25). The tubular epithelial lining of the kidney tissues exhibited mild to severe vacuolation, as well as significant tubular dilation with flat epithelial lining and slight cystic tubular dilation containing homogenous material. Also provide convincing evidence of extensive tubular necrosis (21, 26). In view of the possible damage that the bacteria might inflict in the form of infections, a number of investigations and publications have started looking into alternative methods to reduce the detrimental consequences of different microbial activity

CONCLUSION

The results revealed significant increase in liver function enzymes like (AST, ALT and ALP) and renal function test (blood urea and creatinine) after experimental infection of rabbits by pathogenic *P. vulgaris* bacteria. Postmortem changes examined revealed significant changes in lung, liver, intestine, kidney and heart tissues.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

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