Study of the correlation between levels of TNF-α and MCP-1 in plasma and tissues of rats infected with *Pseudomonas aeruginosa*

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Abstract

For the importance of the infection with *Pseudomonas aeruginosa* which result in serious illness and the complications that may exceed more than one organ of the body, this study was carried using laboratory animals, rats as a model for the mammals' animals to detect the levels of TNF-α and MCP-1 in plasma and tissues and the histopathological effects that can be caused by these bacteria in several organs, lung, liver, spleen, kidney and heart. Methods: In this study, we used 24 rats, 12 untreated (negative) group and 12 treated group that injected intraperitonally with *Pseudomonas aeruginosa* suspension for two periods (1 and 7 days). At the end of experimental period, animals were sacrificed, blood samples were collected and dissected each of lung, liver, spleen, kidney and heart to determine the level of TNF-α and MCP-1 in the plasma and tissues in addition to investigate the histopathological changes. Results: Rats infection with *Pseudomonas aeruginosa* caused a significant increase in the levels of TNF-α and MCP-1 in the plasma and studied organs tissues in comparison with negative rats. In addition to investigate the positive correlation between TNF-α and MCP-1 levels in both plasma and tissues. Light microscopic examination appeared the structural changes in all studied organs of animal infected with *P. aeruginosa* for two periods and this change was more acute in 7 days of treated compared with untreated groups, where have normal structure. In lung, infiltrations of inflammatory cells in addition to necrosis and hemorrhage of respiratory units. Liver sections characterized by irregularity architecture structure with congestion of central vein. Also, we proved abnormal texture of spleen tissue and proliferation in central germination of white pulp. In addition to dilation in parts of urinary duct in kidney, including glomerular collapse, infiltration of lymphocytes. For heart, there is a disorganization of myocardium tissues. While all organs slices of negative groups were normal. Conclusions: We conclude from the results of the current study that the infection with *Pseudomonas aeruginosa* can elevate inflammation in most organs of the body by induce over expression of TNF-α.
and MCP-1, which is reflected negatively on the performance of the function and thus the overall health of the body.

**Key words:** Pseudomonas aeruginosa, TNF-α, MCP-1, lung, liver, spleen, kidney, heart

**ABBREVIATIONS**

TNF-α: Tumour necrosis factor alpha, MCP-1: Monocyte Chemoattractant Protein-1, (IL)-6:Interleukin-6, MIP-2 :Macrophage-inflammatory protein-2, TLR4:Toll- like receptor -4, g :Gram, C˚: Centigrade, rpm :Rotation per minute,IP: Intraperitoneal, CFU/mL :Colony-forming units per milliliter, h:Hour, d : day, EDTA: Ethylene diamine tetra acetic acid, ELISA: Enzyme-linked immunosorbent assay, BPS :Bovine puffer solution, μm: micrometer, H&E :Hematoxylin and eosin, ANOVA: Analysis of variance, DNA: Deoxyribonucleic acid.

**INTRODUCTION**

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a type of Gram-negative bacteria [1]. It is an opportunistic pathogen for human capable of causing acquired infections in the hospital which include acute and chronic infections that threatened to the lives of people, especially in patients with burns, wounds and inflammation of the cornea or patients with planting medical devices, for example, urinary catheters and ventilators [2]. Tancrede and Andremont [3] noted that *P. aeruginosa* is one of the most common gram-negative bacteria translocations in blood; this facilitates tissue access, as with other pathogens, *P. aeruginosa* experiences the effects of oxidative stress caused by the inflammatory response due to phagocytic cells and to counter this natural immune response [4].Infection with *P. aeruginosa* resulted in an increasing in the number of inflammatory cells, expression interleukin (IL)-6 and tumor necrosis factor (TNF)-α that lead to severe inflammation [5]. TNF-α is a pro-inflammatory cytokine that has multifunction's, act as mediators of inflammation secreted by immunoregulatory in response to any injury, inflammation, or infection [6] therefore Cytokines play a major role in innate and adaptive immunity because it contributes to the host's defense mechanisms against the bacterial invasion in its cells [7]. TNF-α has a major role in the evolution and progression of *P. aeruginosa* septic shock, In another word, TNF-α participates directly in the regulation of immune homeostasis [8]. MCP-1 has an important role in mediating of bacterial killing via macrophages [9]. In addition, it considers a direct neutrophil chemoattractant and indirectly regulates other chemokines expression as MIP-2 and KC and cytokines such as IL-6 and TNF-α so will cause neutrophil trafficking [10]. Many intracellular events are triggered when macrophages contact with *P. aeruginosa*, resulting in pro-inflammatory responses., including the production of cytokines, one of these TNF-α [5 ], which participate in cellular signal transduction pathways from which up-regulation of MCP-1 that activate MAPKs [11].

*P. aeruginosa* has different virulence factors and mechanisms that enable them to penetrate and colonize the tissue and the pathogenicity of bacteria depends on the potent of these virulence factors [12]. This, in turn, is reflected on the growth and survival of bacteria and colonization of host tissues which lead to cause injuries of tissue and
debilitating of the immune defence [13]. Several studies have been conducted on laboratory animals to determine the effect of the infection with *P. aeruginosa* or its virulence factors. Woods *et al.* [14] demonstrated that intratracheal administration with *P. aeruginosa* exoenzyme S results in damage of rat lung tissues caused in necrosis of bronchial and capillary endothelial cells and pneumocytes. The study by Saroj *et al.* [15] revealed that mice treated with *P. aeruginosa* exotoxin A caused in renal infection. Baraj and Al-Mathkhury [16] proved that *ip* injection of rats with *P. aeruginosa* DNA resulting in a histopathological change in the spleen including an increasing number of the follicle in the germinal centre in addition of apoptotic cell residues marginal zone of splenic tissue. In addition, The study by Yassein [17] showed the intraperitoneal injection of rats with *P. aeruginosa* DNA caused histopathological change in liver tissue that including infiltration of inflammatory cells and losing architecture of the liver. So, this study aimed to use the rats as a model for the mammals' animals to investigate the effect of infection with *P. aeruginosa* by estimation the level of TNF–α and MCP-1 in plasma and tissues of several organs; lung, liver, spleen, kidney and heart in addition to determine the histopathological change in these organs that can be caused.

**MATERIALS & METHODS**

**Isolation and identification of microorganism**

Clinical isolates of *Pseudomonas aeruginosa* were collected from the sputum of a patient with cystic fibrosis who is visiting the department of Thoracic Diseases at Educational Al-Diwaniyah Hospital. Using plates of MacConkey agar and citramide agar (Himedia, India) as media for bacterial growth that incubated at 37°C for 24 hours, bacterial isolates have been identified via routine laboratory methods according to Holt *et al.* [18].

**In vivo study**

**Animals**

Twenty four of adult white rats (Rattus norvegicus) weighing 200-250 g with age about 8-9 weeks were obtained from the college of Veterinary Medicine, University of Al-Qadisiyah and housed bred in the animal house of Science college, University of Al-Qadisiyah. All animals were permitted to adaptation for 1 week after arrival and they received free access to standard diet and water.

**Induction of rat's infection with *P. aeruginosa***

*P. aeruginosa* was grown in the tryptic soy broth(Sigma-Aldrich) at 37°C for 24 h, then the concentration of growth adjusted to 0.5 by the optical density at 600nm(OD6000), after that the bacterial culture was centrifuged at 8000 rpm for 5 min. The pellet of bacterial cells was washed with a physiological solution and centrifuged again at 8000 rpm for 5 min, after that resuspended of the cells pellet in 10 mL physiological solution, to determine the bacterial cell numbers, the turbidity of prepared suspension was adjusted to (1×10⁸ CFU/mL) after serial dilutions which then used to inject intraperitoneal (ip ) into experimental animal[19].

**Experimental Design**

The experimental animals were divided into two equal groups that contain 12 rats each group and each group assigned into two subgroups (6 rats per subgroup) as following:
1- Positive groups (as infected): included (12 rats), in this groups, all animal were infected by Intraperitoneal injection (ip) with 1×10⁸ CFU/mL of *P. aeruginosa*. After the induction of infection, the animals left two periods until sacrificed (figure 1) as follow:
- Positive Subgroup 1: 6 rats injected (ip) with dose of (1×10⁸ CFU/mL) *P. aeruginosa* and sacrificed after 1 day.
- Positive Subgroup 2: 6 rats injected (ip) with dose of (1×10⁸ CFU/mL) *P. aeruginosa* and sacrificed after 7 days.

2- Negative groups (as control): included (12 rats), in this groups, all animal were free of *P. aeruginosa*. Also, it has been divided into two periods (6 per group) as negative subgroups (for 1 and 7 days).

**Sacrifice animals**

The animals were dissected after the end of each experimental period, blood samples were collected directly by heart puncture and keeping in EDTA tubes until centrifugation and separation of plasma to determine the concentration of TNF-α and MCP-1. The organs (lung, spleen, liver, heart and kidney) were removed immediately for estimation of TNF-α and MCP-1 levels and histopathological studies.

**Estimation of TNF-α and MCP-1**

ELISA technique have been used to determine TNF-α and MCP-1 levels in plasma and tissues samples by following the manufacturer's instructions. Rat TNF-α and MCP-1 ELISA kits were supplied by Elabscience Biotechnology Inc.

To prepare homogenized tissues, BPS (PH 7.4) have been used and homogenized with homogenizer in an ice, then centrifuged at 10,000 × rpm/minute for 30 minute by using cooling centrifuge. TNF-α and MCP-1 levels in the supernatant of the tissues samples homogenate and plasma were assayed by using ELISA kits.

**Histological study**

The specimens of rats tissues (lung, spleen, liver, heart and kidney) after keeping in 10% formalin were dehydrated, then followed by embedding in wax of paraffin followed by paraffin block sectioned into (4-μm thick). Hematoxylin and eosin (H&E) have been used to staining the dewaxed sections[20].

**Data Analysis**

Data of plasma and tissue TNF-α and MCP-1 levels were statistical analyzed using Graphpad prism 7. We used Two-way ANOVA-ordinary--two data set to determine significant different, then followed by Tukey's multiple comparisons test to comparison among groups. Linear correlation between TNF-α and MCP-1 levels in plasma and tissues were done by using computed Pearson Correlation Coefficient, Confidence interval at 95%.

**Figure (1): Schematic diagram explain time of infection and organ collections**
RESULTS

Concentration of TNF-α and MCP-1 in plasma and tissues

The results of measuring of TNF-α and MCP-1 levels in plasma are shown in (figure 2). The levels of TNF-α revealed significant increases (P<0.0001) in positive groups for 1d. and 7d. in comparison with the negative groups and this increase was higher in positive group for seven days compared with positive group for one day (figure 2 a). The results of the plasma concentration of MCP-1 (figure 2 b) demonstrated significant increases (p<0.05) in positive group for 7 days compared with the negative group in the same period and positive groups for 1 day.

From the results of the association between levels of TNF-α and MCP-1 in plasma of infected rats with *P. aeruginosa* (positive groups) for one day and seven days (figure 3), it has been revealed there is no significant correlation (p > 0.05) between TNF-α and MCP-1 in plasma in period one day (r=0.3314) as shown in (figure 3 a). While, in the period seven days, the results confirmed a positive significant correlation (p < 0.05) between levels of TNF-α and MCP-1 in plasma (r=0.8142) in (figure 3 b).

Figure (4) illustrates the effect of infection with *P. aeruginosa* on the levels of TNF-α and MCP-1 in the tissues of the lung, liver, spleen, kidney and heart. The TNF-α concentration significantly increased in studied tissues. In the lung and liver tissues (Figure 4 a and b) respectively, the levels of TNF-α increased significantly in the positive groups for 1 day (p < 0.05) and for 7 days (p < 0.01) in comparison with the negative group in the same period.

In spleen tissues (Figure 4 c), the increment of TNF-α levels in positive groups for two periods (1d and 7d) was at a significant level (p<0.0001) in comparison with the negative group in the same period, in addition, there was significantly different (p<0.05) in TNF-α levels of the positive group for 7 days in comparison with the positive group for 1 day (Figure 4c ). In the kidney, the results recorded significant increases in the TNF-α levels (p<0.01) in the positive group for 7 days in comparison with the negative group and positive group for 1 day. On another hand, there was no significant difference (p>0.05) in the concentration of TNF-α in rat's kidneys in the positive group for 1 day in comparison with a negative group (Figure 4 d).

While the results were not recorded any significant differences (p >0.05) in the levels of TNF-α in the myocardial muscle of positive groups for a period of one day and seven days (Figure 4 e).

The results of MCP-1 levels in studied tissues (figure 4 f, g, h, i and j) demonstrated arise in MCP-1 levels in positive rats groups that injected *P. aeruginosa* are shown in figure (4). The MCP-1 levels increased significantly in the positive group for 7 days in tissues of lung, liver (p<0.05) in (figure 4 f and g), spleen, heart (p<0.01) in (figure 4 h and j) and kidney (p<0.001) in (figure 4 i). On another hand, the increase in MCP-1 concentration did not reach the level of significance (p>0.05) in positive groups for a period of one day in tissues of lung, liver, spleen and kidney. While the results of MCP-1 concentrations confirmed a significant increase (p<0.05) in cardiac tissues in positive groups for one day and seven days (p<0.01) as shown in figure 4(j).

Results of the association between levels of TNF-α and MCP-1 in organs tissues of positive rats groups that injected *P. aeruginosa* for one day and seven days are
shown in (figure 5). In pulmonary tissues, we demonstrated a positive significant correlation between TNF-α and MCP-1 in positive group for period one day ($r=0.9081, \ p < 0.05$) in (a) and for seven days ($r=0.9862, \ p < 0.001$) in (b).

In liver tissues the result recorded positive significant correlation ($p < 0.001$) between TNF-α and MCP-1 in liver in period one day ($r=0.9831$) in (c). Also there was positive significant correlation ($p < 0.05$) between TNF-α and MCP-1 in liver in period seven days ($r=0.8289$) in (d).

Correlation coefficient between levels of TNF-α and MCP-1 in spleen is positive significant correlation ($p < 0.05$) for one day ($r=0.822$) in (e) and for seven days ($r=0.8871$) in (f).

Also, the results confirmed a positive significant association ($p < 0.01$) between the levels of TNF-α and MCP-1 in kidney's positive group for one day ($r=0.9611$) in (g). In the period of seven days, there is positive significant correlation ($p<0.05$) between levels of TNF-α and MCP-1 in kidney ($r=0.8842$) in (h).

In cardiac tissue, a positive significant association ($p <0.05$) is observed between the levels of TNF-α and MCP-1 in positive rats group for one day ($r=0.8229$) in (i), while there is no significant association ($p>0.05$) between the levels of TNF-α and MCP-1 in heart of positive group for seven days ($r=0.577$) in (j).

The figure (6) shows comparison of concentrations TNF-α in tissues of lung, liver, spleen, kidney and heart in the rats groups that injected ip with *P. aeruginosa* (positive groups) for two periods (1 and 7 days). The results of the statistical analysis indicated to a significant increase in the levels of TNF-α in tissues of lung and spleen for one day and seven days in comparison with its concentration in tissues of liver, kidney and heart in the same period. This increase was more significant ($p<0.0001$) in spleen tissues than pulmonary tissues. The results of comparison of concentrations MCP-1 in tissues of lung, liver, spleen, kidney and heart in the positive rats groups revealed significant increase ($p<0.0001$) in the tissues of lung, liver and spleen for two periods (1 and 7 days) compared with kidney and heart in the same period. On another hand, there is no significant different ($p>0.05$) among the levels of MCP-1 of lung, liver and spleen tissues for two periods (figure 7).

![Figure (2). Concentration of TNF-α (a) and MCP-1 (b) in plasma of rats injected ip with *P. aeruginosa* (positive group) compared with control (negative group) for two period (1 and 7 d). (* *represents significant different at level ($P<0.05$), **** represents significant different at level ($P<0.0001$) and values represents means ± standard error](image-url)
Figure (3) correlation between concentration of TNF-α and MCP-1 in plasma of rats injected ip with P. aeruginosa (positive group) for two periods: 1 day (a) and 7 days (b).
Figure (4) concentration of TNF-α and MCP-1 in studied tissues of rats injected ip with P. aeruginosa (positive group) compared with control (negative group) for two period (1 and 7d). TNF-α of lung(a), TNF-α of liver(b), TNF-α of spleen(c), TNF-α of kidney(d), TNF-α of heart(e), MCP-1 of lung(f), MCP-1 of liver(g), MCP-1 of spleen(h), MCP-1 of kidney(i) and MCP-1 of heart(j).

* represents significant different at level (P<0.05), ** represents significant different at level (P<0.01) *** represents significant different at level (P<0.001) **** represents significant different at level (P<0.00001) and values represents means ± standard error.
Figure (5): Correlation between concentration of TNF-α and MCP-1 in studied organs tissues of rats injected ip with P. aeruginosa (positive group) for two periods: 1 day and 7 days.

Figure (6). Comparison of concentration of TNF-α in tissues of lung, liver, spleen, kidney, and heart of positive groups for two periods (1 and 7d). Values represent mean±SE. Different letters indicated significant differences among organs, similar letters refer to non-significant among organs. In period of one week, lung vs. liver (**p=0.0038), lung vs. spleen (****p<0.0001), lung vs. kidney (**p=0.0018), lung vs. heart (**p=0.0017), liver vs. spleen, spleen vs. kidney, and spleen vs. heart (****p<0.0001). In period of two weeks, lung vs. liver ( **p=0.0057), lung vs. kidney (*p=0.0155), lung vs. heart (****p=0.0010), lung vs. spleen, liver vs. spleen, spleen vs. kidney, and spleen vs. heart (****p=0.0001).

Figure (7). Comparison of concentration of MCP-1 in tissues of lung, liver, spleen, kidney, and heart of positive groups for two periods (1 and 7d). Values represent mean±SE. Different letters indicated significant differences among organs, similar letters refer to non-significant among organs. A probability level of the significant differences among all organs (** **p=0.0001).
Histopathological study

Microscopy examination of rats' lungs in negative groups, showed normal texture of lung tissue, in which, pulmonary alveoli have single epithelial layer with normal bronchial epithelial (Figure 8 A and B). While rats' lungs from positive group showed that the infection with *P. aeruginosa* for two periods (1 and 7 days) investigated histopathological changes included thickness in the epithelial layer of pulmonary alveoli therefor increase distances between alveolar sacs, congestion and necrosis in the pulmonary tissue with weak and desquamation in the lining of the lung epithelial and infiltration of inflammatory cells (Figure 8C and D) and this histopathological changes are more in rats that injected with *P. aeruginosa* for seven days (Figure 8D).

No histopathological changes were investigated in the liver of negative rats, normal architecture of liver and hepatocytes appear regular around central vein (figure 9 A and B). While the histological sections examination of infected rat's liver injected *ip* with *P. aeruginosa* showed loss of architecture of liver tissues, degeneration of hepatocytes, sinusoid dilation, congestion with accumulation of neutrophil in central vein (figure 9 C&D).In addition to slight proliferation of kupffur cells in rat's liver infected *ip* with *P. aeruginosa* for 7 days (Figure 9D).

Spleenic tissue that obtained from rat infected with *P. aeruginosa* for (1 and 7 day) revealed histopathological changes included abnormal texture of splenic tissue, also degenerated cells in red pulp hyperplasia in germinal center and fused of white pulp to red pulp (Figure 10 C&D). In addition to release of hemosiderin in the red pulp in comparison with negative groups, which no histopathological changes in texture of splenic tissue have been recorded (Figure 10 A&B).

No abnormal structure in kidney have been shown in negative groups as shown in (Figure 11 A&B). While histological sections of rats infected (ip) with *P. aeruginosa* for two periods (1 and 7 days) investigated inflammation with infiltration of inflammatory cells with necrosis and congestion in urinary tubules (Figure 11 C&D). In addition to dilation of urinary tubules, collapsed of glomeruli and congestion of glomerular capillaries have been shown in rats infected (ip) with *P. aeruginosa* for seven days (Figure 11 D).

The cardiac muscle sections of the rats infected with *P. aeruginosa* revealed abnormal architecture of cardiac muscle. In rats infected with *P. aeruginosa* for 1day showed disorganization of myocardial fibers with inflammation and numerous focal (vacuolation) in (Figure 12C). Myocardial fibers of rats infected with *P. aeruginosa* for 7 days determined sever degeneration with multi vacuolation and infiltration of mononuclear cells (Figure 12D). While the negative subgroup showed arrangement of myocardial fiber architecture with multinucleated (Figure 12A and B).
Figure (8): Histopathology of pulmonary tissue of negative subgroup as control (A&B) and positive rats that injected (ip) with \textit{P. aeruginosa} for two period 1 and 7 days (C&D). Negative subgroup with normal texture of lung tissue (A and B for 1 and 7 d). Inflammation with thickness in epithelial layer of pulmonary alveoli (white arrow). Sever damage in pulmonary units, infiltration of inflammatory and branchial haemorrhage (black arrow) (E&H, stain 400X).

Figure (9): Histopathology of liver tissue of negative subgroup as control (A&B) and positive rats that injected ip with \textit{P. aeruginosa} for two period (1 and 7 day). Negative subgroups with normal architecture of liver tissue (A and B for 1 and 7 d). Loss of architecture of liver, expansion of sinusoid (black arrows), necrosis and congestion in liver tissue (white arrows) (in rats infected for 1 and 7 day C & D). Increase of kupfur cells (head arrow), congestion and accumulation of neutrophil in central vein (in rats infected for 7 day D) (E&H, 100X).
Figure (10): Histopathology of splenic tissue of negative subgroup (as control) and positive rats that injected (ip) with *P. aeruginosa* for two period (1 and 7 days). Negative subgroup with normal texture of splenic tissue (A and B for 1 and 7 d.). Degenerated cells in red pulp (R), hyperplasia in germinal center (G) and fused of white pulp (W) to red pulp (C and D). Released of hemosiderin in red pulp (white arrow) (E&H. 100X).

Figure (11): Histopathology of kidney tissue of negative subgroup (as control) and positive rats that injected (ip) with *P. aeruginosa* for two period (1 and 7 days) in (C&D). Negative subgroup with normal structure of kidney tissue (A and B for 1 and 7d.). Inflammation with infiltration of inflammatory cells and congestion in urinary tubules (black arrows) in (C&D). Collapsed of glomeruli with congestion of glomerular capillaries (blue arrows) (C&D). (E&H. 100X).
Discussion

*Pseudomonas aeruginosa* is scattered everywhere and it has the ability to survive under different conditions as affecting animal and plant, in addition to it is pathogenic cause of the human where causing serious inflammations, especially immune deficiency of patients with cancer, burns and cystic fibrosis [21]. Therefore, it can attack any tissue in the body trapped by immunodeficiency. *P. aeruginosa* is a type of negative bacteria that affects any part of the body, it can cause ear and eye infections, urinary tract infections, intra-abdominal infections, skin and soft tissue infections [22]. One of the most important reasons for the success of bacteria in the invasion of host tissue is its production of toxins and extracellular enzymes, which are important factors in ferocity and persistent. Two extracellular protein toxins are secreted by *P. aeruginosa*, which include exoenzyme S and exotoxin. Exoenzyme S affects the function of the phagocytic cells in the blood and organs, thus facilitating the spread and invasion of *P. aeruginosa* of tissue. While exotoxins inhibit protein synthesis in eukaryotic cells, which cause systemic and local diseases [23]. Cytokines that produced by macrophages play an important role in the pathogenesis, TLR4 recognizes and binding with *P. aeruginosa* to initiates host immune responses resulting in expression of pro-inflammatory cytokines such as TNF-α, this mechanism by which infection with *P. aeruginosa* leads to increase protein levels of TNF-α in the host's body [24]. Many studies have proved that TNF-α can stimulate the expression of adhesion molecules in endothelial which facilitate neutrophils migration to inflamed pulmonary tissues [25]. TNF-α up-regulates expression of MCP-1 protein [11]. As known MCP-1 is a direct neutrophil chemoattractant [10]. Therefore it is evident that
increased TNF-α is accompanied by an increase in the levels of MCP-1, this may be explain the reasons for the significant increase in the levels of TNF-α and MCP-1. This is supported in our study by the results of the correlation between levels of TNF-α and MCP-1, as the increase in plasma level of MCP-1 was accompanied by the increase in plasma level of TNF-α. The finding of increasing pro-inflammatory mediators levels for 7 days rather than one day in plasma and tissues of rats after infected with P. aeruginosa refer to increase inflammatory response period and may be an evidence to probability for conversion of acute inflammation to subacute or even to chronic inflammation and this get back to the extreme pathogenicity of this bacteria because it has many virulence factors that enable it to withstand host defenses [26]. The results of our study are compatible with Epelman et al. [27] who confirmed that P. aeruginosa Exoenzyme S induces increase expression proinflammatory protein cytokines and chemokines such as TNF-α and MCP-1.

Furthermore, the results of histological examination of the studied organs in this study were observed increase the infiltration of inflammatory cells, this an indication to stimulate the immune response of the host's tissues that include varies intracellular signal pathways, their end result increased in the production of cytokines and chemokines. The increase in the levels of TNF-α and MCP-1 can cause alternation in organs texture [28] noted the increase of cytokines in acute inflammatory response resulting in systemic alterations such as hepatic dysfunction and myocardial defect. In addition, previous studies have proven that Gram-negative bacteria toxin can induce shock via activating monocytes which release TNF-α and other cytokines in pro-inflammatory response and organ injury [29], this suggests that histological change in our study possible due to systemic inflammatory responses that supported by results of the levels of TNF-α and MCP-1 in plasma in addition to its levels in studied tissues. Many studies have shown similar effects, Matsushit et al. [30] noted that treated of rats with endotoxin from P. aeruginosa caused increment of TNF-α in addition to myocardial injury as infiltration of inflammatory cell and myocardial necrosis. On another hand, the maturation of macrophages occurs during the migration of monocytes in the bloodstream into cardiac muscle in response to inflammation, then begin release proteolytic enzymes resulting in myocardial damage [31]. The action of MCP-1 during recruitment of monocyte activation of specific adhesion molecule, which caused a myocardial injury [32], this explains myocardial damage when a bacterial infection occurs.

In hepatic tissues, Kupffer cells produce TNF-α as a proinflammatory response [33] and consider mediator to apoptosis of hepatocytes. The proliferation of Kupffer cells that observed in the infected liver in present study consistent with increasing levels of TNF-α in liver tissues this indicated to the important roles of Kupffer cells in excessive production of TNF-α and hepatic damage [34]. In the kidney, the production of TNFα in a specific region that correlated with compartment injured, where the most TNF-α produced in urinary tubules and glomerular cells [35]. The result of this study suggest that infection with P. aeruginosa initiated proinflammatory responses as a part of innate immunity against P. aeruginosa invasion which requires contribution of host factors such as TNF-α, MCP-1 and increased inflammatory cells infiltration that can trigger signals such as the
release of cytokine and chemokine to activate many pathways. The final outcome of it is increasing of host responses, this indicate that organs changes associated with levels of TNF-α and MCP-1 in plasma and tissues. 

The current study proved many histopathological changes in all studies organs (lungs, liver, spleen, kidney and heart). Pulmonary tissues of rats infected with P. aeruginosa showed severe inflammation in pulmonary units and thickness in the epithelial layer of pulmonary alveoli and this possible reflects on the lung compliance, in addition to congestion and necrosis in the pulmonary tissue with weak and desquamation in the lining of the lung epithelial. All these changes due to P. aeruginosa exotoxins, which play an important role in the pathogenicity as they cause leucopenia, circulatory collapse, pulmonary edema, liver infection, necrosis of urinary tubular and edema of pulmonary alveoli, also phospholipase that produce by P. aeruginosa lead to pulmonary necrosis and these exotoxins have the necrotizing effectiveness at the local of colonization of bacteria and ensures the survival of the pathogen longer duration in the host cells, which may cause chronic inflammation. As well as the production of hemolyxin from P. aeruginosa that isolated from respiratory infections, also plays an important role in the bacterial pathogenicity. Rapid neutrophil infiltration in the lung is characteristic of infection with P. aeruginosa and this enhances the immune response to the pathogen. Neutrophils target pulmonary alveoli due to their secretion of cytokine, which is an innate immune response. Our study is compatible with Song et al. who have proved that the infection of mice with P. aeruginosa caused lung injury as it leads to increase increment numbers of pulmonary macrophages that infiltrating the alveolar barriers.

Our study has been found abnormal architecture of liver and hepatocytes in rat infected (ip) with P. aeruginosa in addition to apoptosis of hepatocytes with proliferation of kupffur cells and accumulation of neutrophil in central vein. The inflammatory effects of infection with P. aeruginosa may be due to cytokines and chemokines that produced as pro-inflammatory response to the pathogen. Cytokines and chemokines are secreted by immune cells and other mediators of inflammation. All these products can be involved directly or indirectly in the organic pathogenicity. The proliferation of kupffur cells is a reflection of the activity of these cells resulting from the induction with pathogen, these cells have an important role in homeostasis as they contribute in the acute and chronic responses of the liver to toxic substances and causing of injury of hepatocytes by producing of inflammatory mediators. Our results from this study were in agreement with Yassein. Rat's splenic tissues infected with P. aeruginosa were abnormal texture with hyperplasia in germinal center and fused of white pulp to red pulp. Some studies have shown that the spleen produces splenic substances and inflammatory mediators that cooperate in the bacterial stability in the host and lead to cause the disease. Also, the release of bacterial DNA after lysis of the bacterial cell as a result of the host's defensive response against pathogens causes immune stimulation, resulting in tissue damage. Baraaj and Al-Mathkhury proved peritoneal injection of rats with P. aeruginosa DNA caused germinal center hyperplasia of white pulp, in addition to dead cells debris surrounded by
macrophages marginal zone. Components of the bacterial cell can induced of cytokine secretion in *P. aeruginosa*, lipopolysaccharide (LPS) can mediated immune stimulation in splenocytes and thymocytes[43].

In rat's urinary tubular tissues infected (ip) with *P. aeruginosa*, inflammation with infiltration of inflammatory cells have been found in addition to dilation and necrosis of urinary tubular. Infected with *P. aeruginosa* caused in extreme inflammation with increment of inflammatory cells and secretion of tumor necrosis factor (TNF)-α and interleukin (IL)-6 [38]. Kidneys are organs that are affected by inflammatory disease and TNFα factor plays an important role in inflammatory cascade that lead to kidney injury (Ernandez and Mayadas[8]. So, Our study is compatible with Shivshetty et al.[44].

In myocardial fibers, there was abnormal architecture that represent as disorganization and inflammation with infiltration of mononuclear cells. TNF-α or bacterial endotoxin can effect on the endocardiac epithelial in addition to stimulate collagen synthesis of cardiac fibroblasts [45-47]. Also (TNF-α) can involve in myocardial injury by mediated it in the pro-inflammatory response [45].

**CONCLUSIONS**

From our present study, we demonstrated that infection with *P. aeruginosa* induces excessive production of TNF-α and MCP-1 in plasma and tissues, which can affect the vital organs such as: lung, liver, spleen, kidney and heart by induced pro-inflammatory response and this lead to infiltration of inflammatory cells reflect on texture of tissues.

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