

Effect of *P. aeruginosa* metabolic products on some hematological, biochemical and histological parameters of male rats

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Abstract

The present study was designed to prove the toxic effects of Effect of *P. aeruginosa* metabolic products on some hematological, biochemical and histological on male rats Forty mature male rats, their age average was about (3-3.5) months and their weight average was about (200-225) gm, were randomly divided into 2 equal groups: treatment group (T) were drenched orally by four concentration of *P. aeruginosa* metabolic products (5,50,500,5000) $\mu\text{l/kg}$ body weight, five animals for each concentration and control treated orally by normal saline in the same concentrations above, also five animals each. The animal treated with one single dose of the previously describe concentrations then left for 15 days. Animals of different groups were sacrificed under light anesthesia one day after the end of treatment and blood samples were taken to determine some physiological, biochemical parameters, also the liver and kidneys samples.

The results revealed many changes due to treatment fluctuated according to the type of studied parameter, as follows: significance increase of WBCs count, serum ALT activity, serum AST activity, serum sugar, while hemoglobin (Hb) and serum triglyceride (TG) concentration shows significance decrease. Also the microscopic examination of the organs shows many histopathological changes due to the treatment such as hemorrhage, extension of central vein, disarrangement of sinusoid, degeneration and hepatocytes necrosis in the liver, while kidneys sections shows epithelial infiltration, simple glomerulus's extension and slight tubular degeneration .

The findings of this study showed that liver function and liver structure severely effected by *P. aeruginosa* metabolic products as well as other parameters due to the treatment.

Introduction

Pseudomonas aeruginosa is saprophytic organism widely distributed in soil, water, and vegetation and is commonly present in moist environment in hospitals, also It is found in the skin of some healthy persons (Pollack, 2000) and it causes diseases in humans with abnormal host defenses. They are motile and rod-shaped measuring about $0.6 \times 2 \mu\text{m}$ (Sleigh & Timbury, 1998). It is Gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains (Forbes *et al.*, 1998). Pseudomonal infections can involve any part of the body, like respiratory tract, central nervous system (CNS), cardiovascular system, ear and eye. Pneumonia and chronic infection of the respiratory tract are the most well known diseases following aspiration of *P. aeruginosa* from the upper respiratory tract or may occur as a result of bacteremic spread to the lungs (Garah, 2001). Pseudomonal gastro- intestinal infections can range from very mild symptoms to severe necrotizing enterocolitis with significant morbidity and mortality, also epidemics of pseudomonal diarrhea can occur in nurseries (Molina, 2001). Bones and Joints may infected with *P. aeruginosa*, and the most common sites of involvement are vertebral

column, pelvis, and sterno clavicular joint. The infection is either contiguous related to penetrating trauma, surgery, or overlying soft tissue infections as in patients with diabetes mellitus or peripheral vascular disease or the infection may be blood-born as in IV drug abusers or patients with UTI (Ryan *et al.*, 1994). Meningitis and brain abscess can be caused either locally from infection in the ear or para nasal sinuses or by hematogenous spread from infective endocarditis, pneumonia or UTI (Garah, 2001). This organism may infect native heart valves in individuals who abuse intravenous drugs and it may infect prosthetic heart valves. The patient may be presented with non-specific symptoms including fever, malaise and may be presented with symptoms of congestive heart failure resulting from systemic spread of septic emboli (Molina, 2001). *Pseudomonas* also has emerged as an important source of burn wound sepsis with involvement of subjacent unburned tissue. Systemic manifestations include fever or hypothermia, disorientation, hypotension, oliguria and ileus (Ryan *et al.*, 1994).

P. aeruginosa is frequently resistant to many commonly used antibiotics (Kerstens *et al.*, 1996). Also demonstrates high intrinsic resistance to multiple classes of antimicrobials, due primarily to a combination of low outer membrane permeability coupled to secondary resistance mechanisms such as β -lactamase activity and efflux systems (Wong *et al.*, 2001). Unfortunately, the availability of antimicrobial drugs for legitimate uses has also led to a level of antibiotic misuse and overuse that could be characterized as “anti biotic pollution”, the misuse of antibiotics includes: improper dose or duration of antibiotics, failure to modify antibiotics based on susceptibility, inadequate access to microbiologic testing and aggressive marketing of broad-spectrum agents and self-prescribing (Mah & Memish, 2000).

Materials and Methods

Pseudomonas aeruginosa:

isolates of *P. aeruginosa* were cultured in nutrient broth media (from B.D.H) for 21 days. After this period the media filtered by filter paper (whattman no.1) (Colle *et al.*, 1996) and maintained in clean sterile tubes until it used in the experiment later.

Animals and Experiment Design

The experiment was performed using forty mature male rats, their age average was about (3-3.5) months and their weight average was about (200-225) gm, were randomly divided into 2 equal groups: treatment group (T) were drenched orally by four concentration of *P. aeruginosa* metabolic products (5,50,500,5000) μ l/kg body weight, five animals for each concentration and control group treated orally by normal saline in the same concentrations above, also five animals for each concentration. The animal treated with one single dose of the previously describe concentrations then left for 15 days.

Blood Collection

The animals were sacrificed under light anesthesia one day after the end of treatment and the blood samples were collected and divided into two portions; the first one was collected into clean heparinized tubes to determine WBCs count (Dacie & Lewis, 1984) and hemoglobin (Hb) (Coles, 1980), while the second one was collected into clean dry centrifuge tubes and left to stand

for 30 minutes at room temperature, then centrifuged at 3000rpm for 10 minutes and serum samples were stored in clean tubes at -20°C till used for assay of AST, ALT (Reitman & Frankel, 1957) , blood sugar (Trinder, 1969) and TG (McGown *et al.*, 1983).

Tissue Sampling

Liver and kidneys organs were excised from all animals. The organs tissue samples were fixed for 48 hours in buffered neutral formalin (10%). Following this procedure, the tissues were processed for histological sectioning according to the method that described by Luna (1968) then stained with hematoxylin-eosin for routine histological analysis according to Wood & Ellis (1994) method.

Statistical Analysis

The data were analyzed by using F test taking $P \leq 0.05$ as the lowest limit of significant of difference and Duncan's Multiple Range Test was used to identify group responsible for statistical difference through comparison (Scheffler, 1980).

Results

Table (1) shows the physiological profile of the normal and treated rats in addition to the serum biochemical parameters of both groups. The treated group (t1) shows significant increases ($P < 0.05$) in all parameters except in Hb and TG concentration which decreased significantly ($P < 0.05$) as compared to control and the differences were increase with increases of the concentrations.

Table (1) shows the physiological profile of the normal and treated rats besides serum biochemical parameters of both groups.

Parameters Concentration	WBCs ($\times 10^9/L$)	Hb (gm/100 ml)	AST (mg/dl)	ALT (mg/dl)	Sugar (mg/dl)	TG (mg/dl)
C 5	3162 $\pm$ 6.50 A	11.8 $\pm$ 0.3 a	7.3 $\pm$ 0.04 A	8.3 $\pm$ 0.91 a	81 $\pm$ 2.2 a	144.2 $\pm$ 3.16 A
C 50	3167 $\pm$ 1.24 B	12 $\pm$ 0.14 b	7.8 $\pm$ 0.12 B	8.0 $\pm$ 1.01 b	83 $\pm$ 1.45 b	142 $\pm$ 1.5 B
C 500	3165 $\pm$ 8.14 C	11.6 $\pm$ 0.09 c	8.1 $\pm$ 0.01 C	8.4 $\pm$ 0.5 c	86 $\pm$ 0.78 c	143.2 $\pm$ 2.0 C
C 5000	3160 $\pm$ 5.92 D	11.2 $\pm$ 0.84 d	7.2 $\pm$ 0.10 D	8.1 $\pm$ 2.0 d	85 $\pm$ 0.06 d	141.2 $\pm$ 1.72 D
T 5	3205.6 $\pm$ 6.05 A	10.5 $\pm$ 0.02 a	8.40 $\pm$ 0.5 A	9.77 $\pm$ 1.0 A	89.62 $\pm$ 0.01 A	127.25 $\pm$ 5.5 A
T 50	3490.25 $\pm$ 8.10 B	9.05 $\pm$ 0.1 B	9.60 $\pm$ 0.76 B	12.75 $\pm$ 0.77 B	101.72 $\pm$ 0.14 B	118.47 $\pm$ 3.15 B
T 500	5040.25 $\pm$ 23.2 C	7.12 $\pm$ 0.16 C	12.52 $\pm$ 1.4 C	16.70 $\pm$ 2.83 C	123.32 $\pm$ 1.10 C	98.47 $\pm$ 2.5 C
T 5000	6705 $\pm$ 14.9 C	5.4 $\pm$ 0.11 D	18.42 $\pm$ 1.1 D	20.9 $\pm$ 0.89 D	161.25 $\pm$ 2.03 D	85.15 $\pm$ 2.4 D

*Numbers represented averages $\pm$ standard error.

*Capital letters mean that there are significant differences (P<0.05).

Liver examination

In the light microscopical examination of the control group liver tissue samples, central vein surrounded by parenchymal cells which enclosed among them spaces called sinusoids that contain Kuppfer's cells (Fig.1). Whereas group 1(T) samples revealed many severe changes with

P. aeruginosa treatment such as hemorrhage, extension of central vein, disarrangement of sinusoid, degeneration and hepatocytes necrosis (Fig.2).

Kidney examination

Normal structure of kidney cortex present in control group (Fig.3) In the same time, kidney tissue samples of treated rats shows slight structural disturbance as compared to control samples. Observation revealed epithelial infiltration, simple glomerulus's extension and slight tubular degeneration (Fig.4).



Fig.3: Kidney section taken from rat belongs to control group shows glomerulus (black arrow), proximal tubules and distal tubules (blue arrow), (H&E x 500)

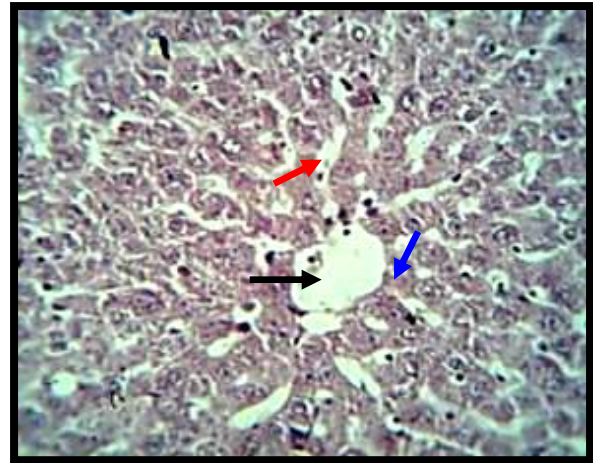


Fig.1: Liver section taken from rat belongs to control group shows central vein (black arrow), parenchymal cells (blue arrow), sinusoids and Kuppfer's cells (red arrow) (H&E x 500)

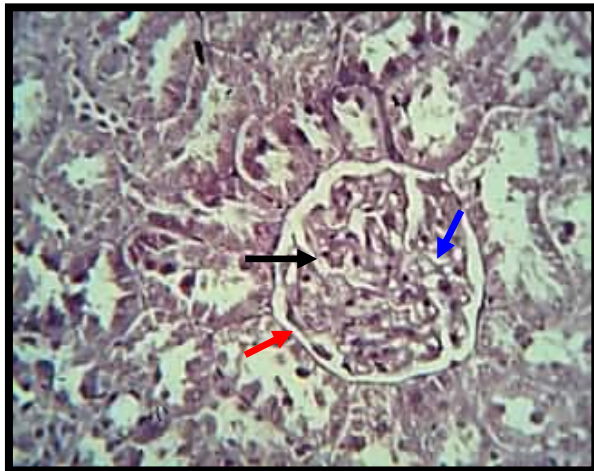


Fig.4: kidney section taken from rat belongs to the group that treated with 5000µl/kg of *P. aeruginosa* metabolic products shows glomerulus extension (black arrow), epithelial infiltration (blue arrow) and degeneration of tubules (red arrow) (H&E x 500)

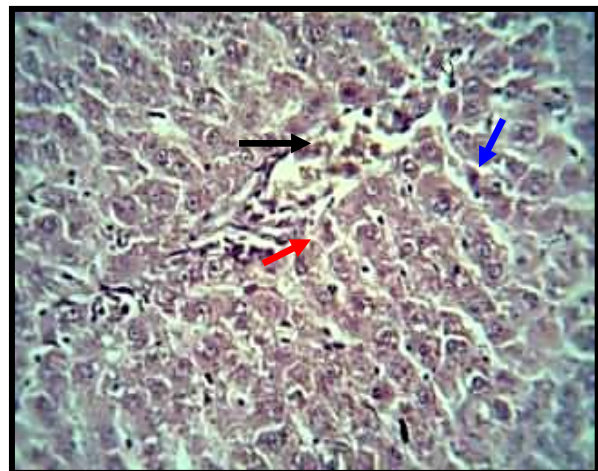


Fig.2: Liver section taken from rat belongs to the group that treated with 5000µl/kg of *P. aeruginosa* metabolic products shows central vein extension and hemorrhage (black arrow), hepatocyte necrosis (blue arrow), disarrangement of sinusoids (red arrow) (H&E x 500)

Discussion

There is no single well defined mechanism that may explain the quantitative and qualitative changes occur in the study parameters as well as organs histology after treatment, since the pathogenesis of pseudomonal infections is complex and is evidenced by the clinical diversity of the diseases related to this organism, and multiplicity of virulence factors it produces (Kerstens *et al.*, 1996). It produce extracellular enzymes and toxins that by break down physical barriers and cause tissues damage (Chiu *et al.*, 2009) (especially the most sensitive organs are those with a high energetic demand and with an active metabolism, such as central nervous system, myocardium and liver), such as exotoxin A (Schumann *et al.*, 1998), exoenzyme S, proteases, cytotoxin, phospholipase C, rhamnolipid, and pyocyanin (Iglewski, 2002). Proteases: These include elastase & alkaline protease. The incidence of production is about 90%. They cause rapid tissue necrosis and destruction which aid the bacteria in tissue invasion i.e. spreading factor. These proteases can dissolve elastin and fibrin, destroy collagen, affect the host physical barriers (Iglewski, 2002).

Furthermore, the liver, is an organ with high metabolic dynamics, also it has been shown that the liver is the prime location for removing foreign substance (Jones, 1996), this may explain the concentrating damage in liver tissue. Since kidneys are the major excretory route for excretion of xenobiotics , this organ is particularly exposed to untoward toxic effects. Among the mechanisms discussed in the pathogenesis of *P. aeruginosa* renal damage effect on renal tubules epithelium due to progressive inflammation.

P. aeruginosa sepsis induced a significant production of tumor necrosis factor (TNF) and interleukin-6(IL-6) (Neely *et al.*, 1996) and cytokines, these cytokines included proinflammatory, hematopoietic and immunoregulatory cytokines (Kemacki *et al.*, 1998). Excessive production of these cytokines is likely to be the main cause to increased total count of WBCs in treated rats in this study. Besides the excessive production of these substances cause progressive oxidative stress in cells due to increased free radicals which attack erythrocytes and destroyed it leading to impaired its functions and decrease Hb concentration (Guyton & Hall, 1997).

The enzymes are biochemical macromolecules that control metabolic process of the organisms, thus by estimating the enzyme activities in an organism, we can easily identify disturbances in its metabolism (Arneson & Brickell, 2007). ALT and AST enzymes are located in various structure of the cells, but concentrated mainly in liver cells also they found in the kidneys (Kachmar & Moss, 1982). Increased activity of ALT and ALT is a sensitive sign of impaired organs membrane and well indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane, and membrane transport system. Such damage to these has been reported in various previous studies (Liu, 1974). Decrease TG concentration may be explain by the impairment of liver structure that observed in this study, since liver constitute the lipase enzymes that hydrolyzes TG to glycerol and fatty acids (Guyton & Hall, 1997; Mayes, 2000) thus, destruction of the liver cause leakage of this enzymes to the blood stream and cause decrease TG concentration as a result.

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تأثير المنتجات الايضية لـ *P. aeruginosa* على بعض المعايير الدموية والكيموحيوية والنسجية لذكور الجرذان.

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الخلاصة

صممت الدراسة الحالية من اجل التعرف على التأثيرات السمية للمنتجات الايضية لبكتريا *P. aeruginosa* على بعض المعايير الدموية والفسلجية والكيموحيوية لذكور الجرذان . تم تقسيم 40 من ذكور الجرذان، بمعدل عمر تراوح بين (3-3.5) شهراً ومعدل وزنها تراوح بين (200-225) غم، عشوائيا إلى مجموعتين متساويتين: مجموعة المعاملة (T1) والتي جرعت فمويا بأربع تراكيز من المنتجات الايضية للبكتريا (5,50,500,5000) مايكروليتر/ كغم من وزن الجسم وكل تركيز ضم 5 حيوانات ، ومجموعة السيطرة والتي جرعت فمويا بالمحلول الملحي الفسيولوجي بنفس التراكيز المشار إليها في أعلاه وبنفس عدد الحيوانات لكل تركيز. هذه التراكيز أعطيت مرة واحدة فقط ثم تركت لمدة 15 يوما. تم قتل الحيوانات في المجاميع المختلفة بعد تخديرها عند نهاية مدة التجربة بيوم واحد و تم اخذ نماذج الدم لقياس بعض المعايير الفسلجية والكيموحيوية فضلا عن نماذج للكبد والكلية.

أظهرت النتائج عدة تغيرات نتيجة المعاملة تباينت تبعا لنوع المعيار المدروس وكانت كالآتي: ارتفاع معنوي في العدد الكلي لخلايا الدم البيض وفعالية إنزيم AST في المصل و فعالية إنزيم ALT في المصل والسكر في المصل بينما شهد تركيز الهيموغلوبين و الكليسيريدات الثلاثية انخفاض معنوي. كذلك اظهر الفحص المجهرى للأعضاء عدة تغيرات نسجية مرضية نتيجة المعاملة مثل النزف الدموي، توسع الوريد المركزي، عدم انتظام الجيبانيات الكبدية وتنخر وتحلل الخلايا الكبدية، بينما مقاطع الكلى أظهرت حصول تكاثر للخلايا الطلانية وتوسع بسيط للجسيمة الكلوية وتحلل طفيف للنيبيات.

ما تقدم من النتائج يتبين أن للتراكيز المختلفة من المنتجات الايضية لبكتريا *P. aeruginosa* تأثير على الكبد وظيفيا وتركيبيا فضلا عن بقية المعايير المدروسة.