Inhibitory effect of honey on some bacterial infections

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Abstract

The antibacterial profiles of honey were examined against clinical isolates of *Staphyllococcus aureus* and *Pseudomonas aeruginosa*. Agar well diffusion method was used in the antibacterial susceptibility studies. This study revealed that the honey obtained from Agricultural college of Baghdad University was effective against bacteria.The highest inhibitory zone of *S. aureus* reached to 20mm in diameter at 200 mg/ml of honey concentration but not affected on *P. aeruginosa*. Wounds were done in male mice by using shaver and then infected with 0.1ml of both bacterial suspensions with concentration 10^8 CFU/ml.Symptomes of infections were appeared after 24-48 hours. Three concentrations of honey were used (100,200 and 300) mg/ml for treatment of skin infections by using 0.1 ml of honey,also gentamycin ointment and normal saline were used. Symptomes of recovery based on wound healing were noticed after 10,6,9,11 and 15 days for *S. aureus* and 12,8,13,15 and 18 days for *P. aeruginosa* when treated with 100,200 and 300mg/ml of honey,gentamycin and normal saline respectively. 200 mg/ml of honey concentration showed the best concentration for skin infection treatment. This study,therefore, suggest that honey could have strong biocidal effect against *S. aureus* (both *in vitro* and *in vivo*) and against *P. aeruginosa* (*in vivo*),therefore,have the potential effective role in the treatment of skin infection.

Introduction

Wide range of current antibiotics are available for treatment of bacterial infection, but there are still some challenges to be met in microbial chemotherapy.One of the problems is the development of resistance to chemotherapeutic agents due to abuse of these drugs(1).Therefore ,the use of alternative therapies for treatment of antibiotic-resistant bacteria ,which become a major problem ,was giving more interest(2).

Staphylococcus aureus and *Pseudomonas aeruginosa* are considered major contaminants of wounds (3,4,5). Because *S. aureus* is found as normal flora in respiratory tract and on skin ,therefore , it can invade the opened wound causing their purulent(6,7),while *P. aeruginosa* has several virulence factors which play an important role in infections, and alginates ,as well as ,their resistance to wide spectrum of antibiotics(4,5,7).According to that,researchers became to use alternative therapies like medical herbs,plant extracts,for example,Thyme (*Thymus vulgaris L.*), Harmal (*Penganum harmala L.*), Fenugreek (*Trigonella foenum-graecum*) (8),Henna (*Lawsonia inermis*) (9),and also Green tea extracts were used as an antibacterial agents (10).

Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and an- aerobes,gram-positive and gram negatives,and antifungal effect had been observed for some yeasts,as well as species of *Aspergillus* and *Penicillium* (11), as well as the dermatophytes (12).Honey is a sweet food made by some insects using nectar from flowers. The variety produced by honey bees (the genus *Apis*). Although it may be very viscous or even solid at room temperature and even more fluid if diluted with proportionally small volumes of exudates. Honey produced as a food often is not well filtered and may contain various particles in it.Although honey does not allow vegetative form of bacteria to survive, it does contain *Clostridium botulinum* spores and toxins that can cause infant botulism (a life threating paralytic disease) ,so it is safe for children older than 12 months and adults (11).Therefore,honey that has been treated by gamma irradiation is available commercially;this processing kills clostridial spores without loss of any of the antibacterial activity (12,13). It has density of about 1.36 kilograms per liter (36% denser than water) (14).pH of honey is commonly between 3.2 and 4.5.This acidic pH level prevents the growth of many bacteria (15).Honey is a mixture of sugars and other compounds.With respect to carbohydrates , it is mainly fructose (about 38.5%) and glucose (about 31%) , other honey's

remaining carbohydrates include maltose , sucrose , and other complex carbohydrates. Its tiny amounts of several compounds thought to function as an antioxidants including chrysin, pinobanksin , vitamin C, catalase and pinocembrin (16,17).

Honey had been used since ancient times both as a food and as a medicine. Human began hunting for honey at least 10,000 years ago. It is an ancient remedy for the treatment of infected wounds,which has recently been rediscovered by the medical profession, particularly when conventional modern therapeutic agents are failing. It may promote better blood sugar control , also reduce oxidative stress frequently by a larger factor than can be explained by their actual amount,this may be beneficial for diabetics and help improve endothelial function and vascular health.Honey boosts immunity,increased neutrophil count,decreased thrombocytopenia (low platelet count),stabilize hemoglobin level (18).Antioxidants in honey have been implicated reducing the damage done to the colon in colitis (19).According to these advantages of honey,it was used to investigate it's antibacterial activity against *S. aureus* and *P. aeruginosa* .

Materials and Methods

_ Bacterial Isolates

S. aureus and *P. aeruginosa* were obtained from Biology Department of Science college of Baghdad University after their isolation an diagnosis.

_ Concentrations of honey

Honey was obtained from local markets.This honey was obtained from Agricultural college of Baghdad University . Three concentrations of honey were prepared by using sterile disttled water: 100,200 and 300 (mg/ml) .

_ Effect of honey *in vitro*

The antibacterial activity was determined by using agar well diffusion method (20). 10^8 CFU/ml bacterial concentrations were prepared for both bacteria in normal saline then wells were made in nutrient agar. Plates were cultured by using small swab of each bacteria.0.1ml of honey were added to the wells from each concentration.The inoculated plates were incubated at 37C˚ for 24 hrs.

The diameters of inhibition zones were measured for each plate. The standard Ampicillin disc(10 mcg) was used as control for *S. aureus* and standard cefotaxime disc (30 mcg) was used as a control for *P. aeruginosa* by pressing these discs on the cultured plates gently.

_Effect of honey *in vivo*

Thirty male mice were obtained from veterinary medicine college of Baghdad University , and divided into 5 groups for each bacteria and each group composed of three mice.

Group 1 : was treated with 100 mg/ml of 0.1 ml of honey (10 mg/0.1ml).

Group 2 : was treated with 200 mg/ml of 0.1 ml of honey (20 mg/0.1ml).

Group 3 :was treated with 300 mg/ml of 0.1 ml of honey (30 mg/0.1ml).

Group 4 : was control negative which treated with normal saline.

Group 5 : was control positive which treated with Gentamycin Ointment.

These groups were applied for each bacteria.

_ Skin infection

Clipping and shaving were made at the back site of mice until they were injured and infected with 0.1 ml of each bacterial suspension with 10^8 CFU/ml concentration. symptoms were appeared after24-48hrs.from infection.Days required for skin Infectious symptoms disappeare of mice were observsd and compared with control groups.

_Histopathological study

The specimens of skin were fixed in 10% neutral formalin buffer solution till the preparation of histological sections.tissue were embedded in paraffin and several tissue sections were prepared for histological sections were stained with Hematoxylin-Eosin (H&E)stain (21).

Results

_ Effect of honey *in vitro*

Results showed that 200 mg/ml of honey was the best concentration for *S. aureus* inhibition. It showed 20mm of inhibition zone (figure 1) and it was selected to study it's activity in histological sections,while both 100 and 300(mg/ml) gave no inhibition zones.Ampicillin antibiotic gave 15 mm of *S. aureus* inhibition.The second bacteria which was *P. areuginosa* was resistant for all honey concentrations and no inhibition zone was seen when using agar well diffusion method,as well as it showed full resistance for cefotaxime antibiotic.(Table 1).

Table 1: Honey concentrations effect on bacteria *in vitro.*

Honey concentrations (mg/ml)	Diameter of inhibition zones of *S. aureus* (mm)	Diameter of inhibition zones of *P. aeruginosa*(mm)
100	_	_
200	**20**	_
300	_	_
Ampicillin	**15**	_
Cefotaxime	_	_

(-):no inhibition.

Symptoms of skin infection

After wounds were made *in vivo*,the signs of lesions were appeared after 24-48 hrs. for both bacteria ,which characterized by skin inflammation,redness, swallowing,spots of pus and abscesses in the infected area.

_Effect of honey *in vivo*

After treatment with three concentrations of honey,the mice rendered sterile after (10,6,9) and (12,8,13)days from infection for *S. aureus* and *P. aeruginosa* when treated with (100,200,300)mg/ml of honey concentrations respectively. Animals treated with gentamycin and normal saline healed after 11,15 days from infection with *S. aureus*,while animals infected with *P. aeruginosa* required 15,18 days for healing from infection when treated with gentamycin and normal saline respectively.(Table 2).

Table 2 :Days required for recovering of skin infections *in vivo*.

Treatments	No. of days for *S. aureus* recovery.	No. of days for *P. aeruginosa* recovery.
100 mg/ml	10	12
200 mg/ml	6	8
300 mg/ml	9	13
Gentamycin	11	15
Normal saline	15	18

_Histopathological study

Groups of animals which infected with *S.aureus*_ and *P. aeruginosa* were divided into three groups: the first group was infected with *S. aureus* only (without treatment),histopathological section showed inflammatory cells infiltration with fibers in the dermis and extended to subcutaneous tissue (figure 2),and the animals infected with *P. aeruginosa* only (without treatment) showed that the main lesion in the skin characterised by inflammatory cells infiltration mainly nutrophils in subcutaneous tissue (figure 3),and in the epidermal layer together with the dermis,there is congestion of blood vessels as well as sloughing and necrosis of epidermal cells with sever nutrophils infiltration (figure 4).The second group was treated with 200 mg/ml of honey for animals infected with *S. aureus* exhibited granulumatous tissue consist of congested capillary blood vessels and proliferation of fibrocytes and few mononuclear cells infiltration (figure 5), whereas, in animals infected with *P. aeruginosa* , the microscopic section showed no clear pathological lesion in the skin except few mononuclear cells infiltration in the dermis and slightly proliferation of fibrous connective tissue (figure 6).The last group of animals was treated with gentamycin ointment . The animals infected with *S. aureus* , their microscopic examination of skin showed no clear pathological changes except few mononuclear infiltration (figure 7),while the histopathological examination of animals infected with *P. aeruginosa* showed more inflammatory cells infiltered in dermis and subcutaneous tissue (figure 8).

Discussion

Many local studies were done to proove that *S. aureus* and *P. aeruginosa* were the major pathogens of wounds than other kinds of bacteria (22,23,24). *S. aureus* is found as normal flora in respiratory tract and on skin ,therefore,it can invade the opened wound causing their purulent(6,7).This bacteria has many virulence factors like production of hemolysin,hyaluronidase,coagulase,DNase, protease,enterotoxins and protein A(4).One of the studies showed that contamination with *S. aureus* was 60% in skin bacterial infections(25).On the other hand,*P. aeruginosa* is one of the major wounds pathogen because of it's resistance to wide spectrum of antibiotics(4),also this bacteria contains several virulence factors like exotoxin A,production of elastase,protease and hemolysin which play an important role in infection.Infection with this bacteria often considered as nosocomial infection.25% of patients in hospitals are infected with this pathogen(26).

Results obtained from this study showed that 200mg/ml concentration of honey was the best one for treatment of *S. aureus* (both *in vitro* and *in vivo*) and *P. aeruginosa* (*in vivo*).This may refer to the nature of formation of microbial cells which determine the suitable dose for their inhibition.This may explaine that 100,300(mg/ml)were not the optimal concentrations for bacterial inhibition,also pinocembrin (which is unique to honey) that is characterized by it's antibacterial properties(27,28)may be more released in 200 mg/ml of honey than in other concentrations.Honey absorbed water in the infected area,so drying it out because it composed mainly of glucose and fructose that are strongly attract water,so the growth of bacteria and fungi was inhibited because these microorganisms thrive in a moist environment.Secodly,honey contains an enzyme called glucose oxidase that when combined with water,produces hydrogen peroxide which release slowly and act as bacteriocidal agent.

$$C_6H_{12}O_6 + H_2O + O_2 \longrightarrow C_6H_{12}O_7 + H_2O_2$$ (29).

The harmful effects of hydrogen peroxide are further reduced because honey sequesters and inactivates the free ion which catalyses the formation of oxygen free radicals produced by hydrogen peroxide (30).Days of mice healing were detected according to infectious symptoms disappearance.This result may be compatible for that which had been reported from various clinical studies on the usage of honey as a dressing for infected wounds that the wounds became sterile in 3-6 days(31), 7 days (32,33), or 7-10 days (34). Honey also contains antioxidants and flavonoids that may function as antibacterial agent (16,17).Recent research showed that the proliferation of peripheral blood B and T _lymphocytes in cell culture,

release of cytokines , tumor necrosis factor (TNF),alpha inter-leukin-1(IL_1) and IL_6 which activate the immune response to infection were stimulated by honey(35,36).Honey can allow burn wound tissue to heal rapidly,necrotic tissue are replaced with granulated tissue and advancing epithelialisation when honey is used as dressing, which lead to separate of necrotic and gangrenous tissue so that they could be lifted off painlessly (37).As aconclusion from this study,honey can be used as atreatment of some skin bacterial infections at 200mg/ml concentration.

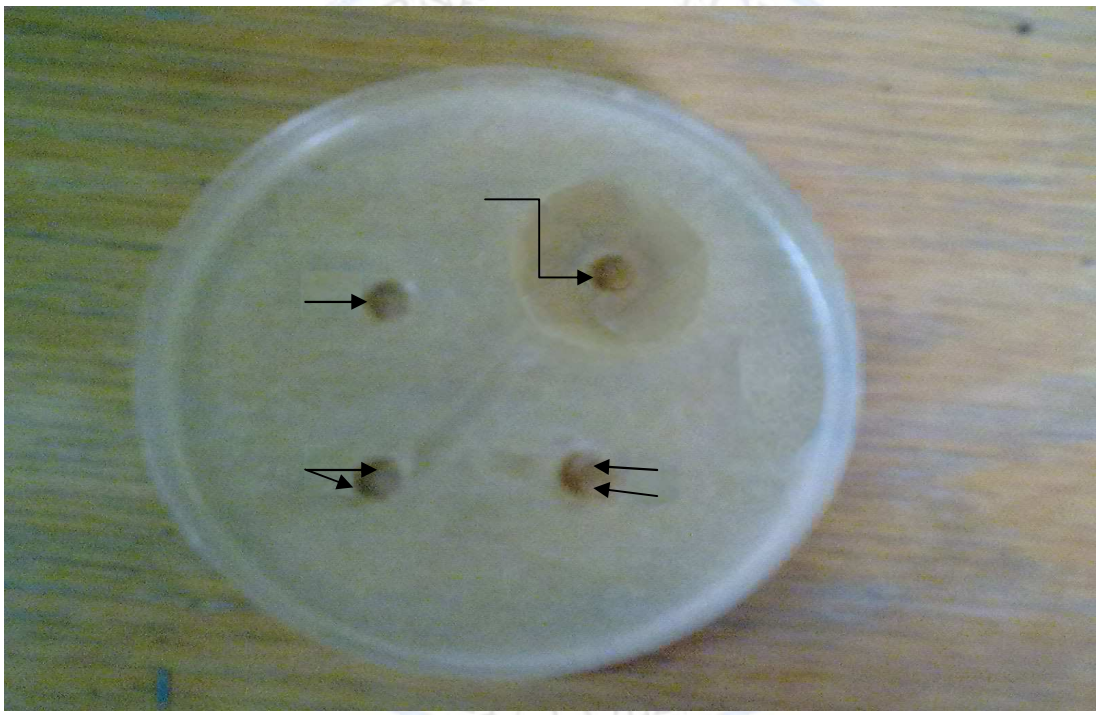

Figure (1):show inhibition zone of *S. aureus* by 200mg/ml of honey.

(→):100mg/ml , (⌐→):200mg/ml , (⇉):300mg/ml , (↘):normal saline.

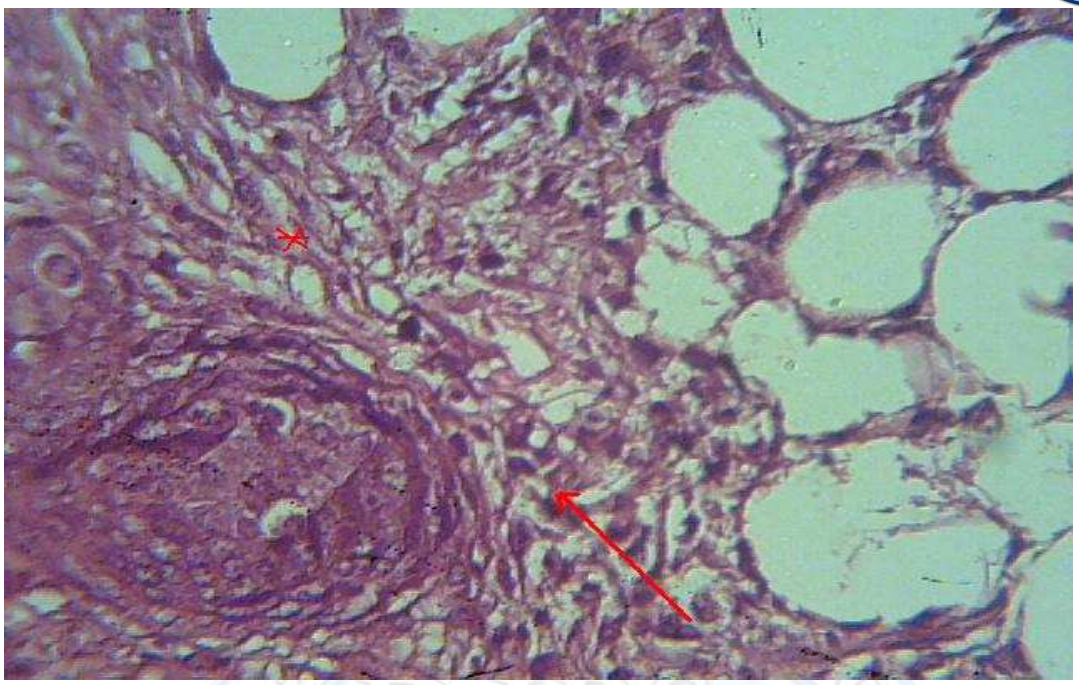

Figure (2): Histological section in skin of an animal after 24-48 hrs. of infection with *S. aureus* show mononuclear cells infiltration in the dermis (arrow) with marked proliferation of fibrous tissue (*) by H & E (400X).

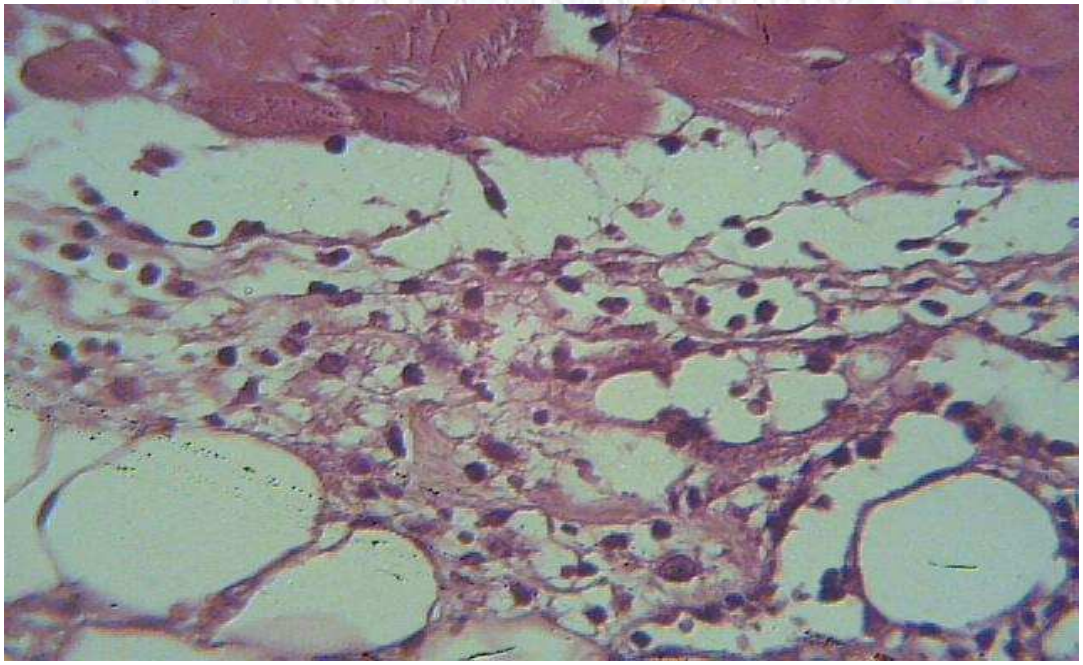

Figure (3): Histological section in skin of an animal after 24-48 hrs. of infection with *P. aeruginosa* show infiltration of nutrophils in subcutaneous tissue.

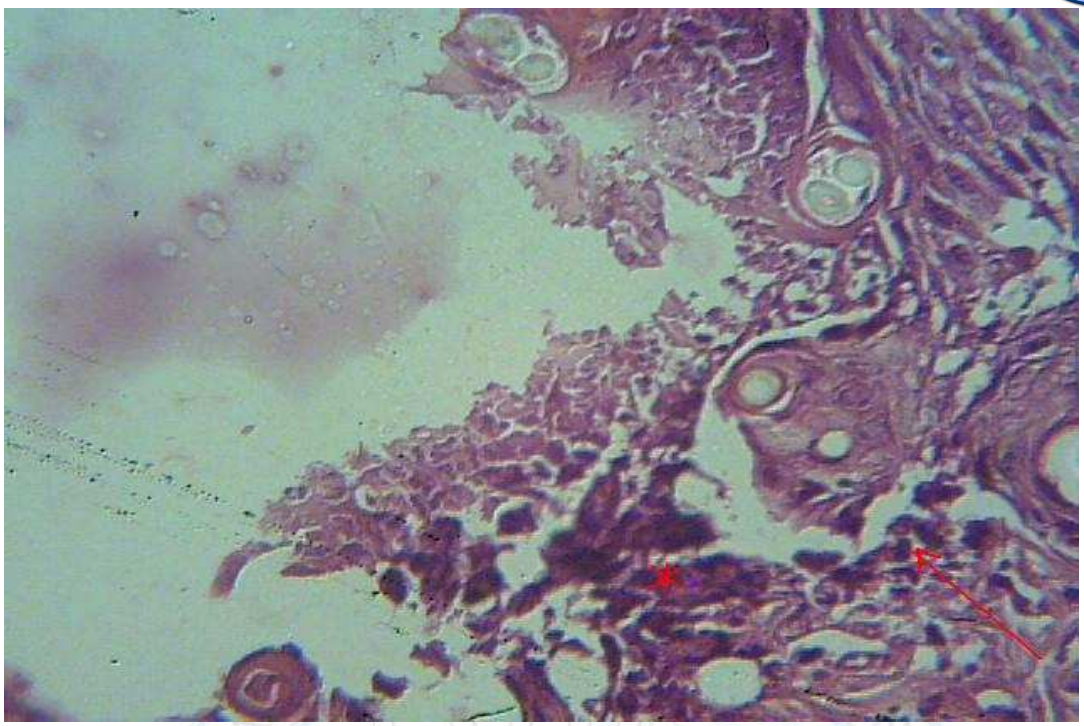

Figure (4): Histological section in skin of an animal infected with *P. aeruginosa* show necrosis and sloughing of epidermis (arrow) with nutrophils infiltration (*) by H&E (400 X).

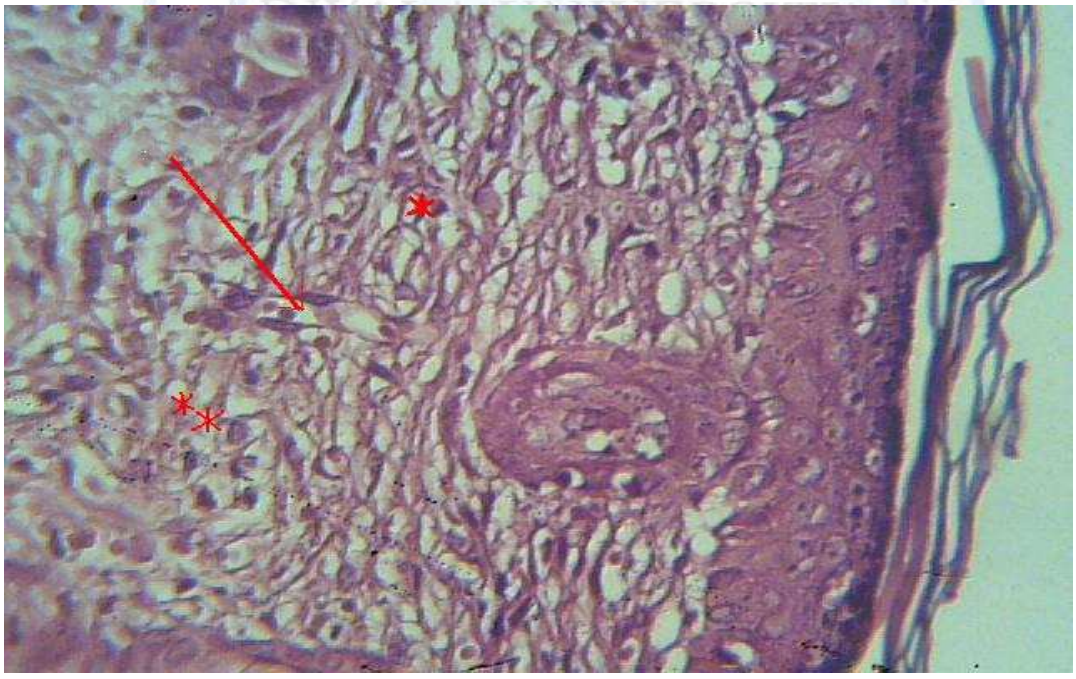

Figure (5): Histological section in skin of an animal infected with *S. aureus* show congested blood vessels (arrow) and proliferation of fibrocytes in the dermis (*) and few mononuclear infiltration (**) by H&E (400 X).

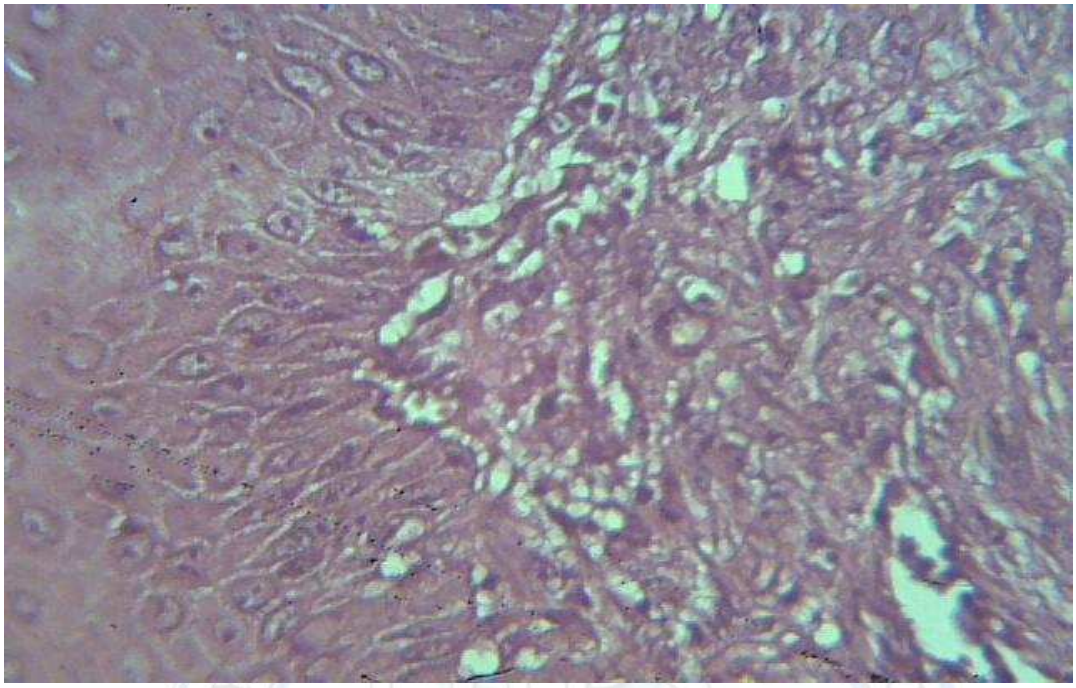

Figure (6): Histological section in skin of an animal infected with *P. aeruginosa* show few mononuclear cells infiltration in the dermis and slightly proliferation of fibrous connective tissue by H&E (400 X).

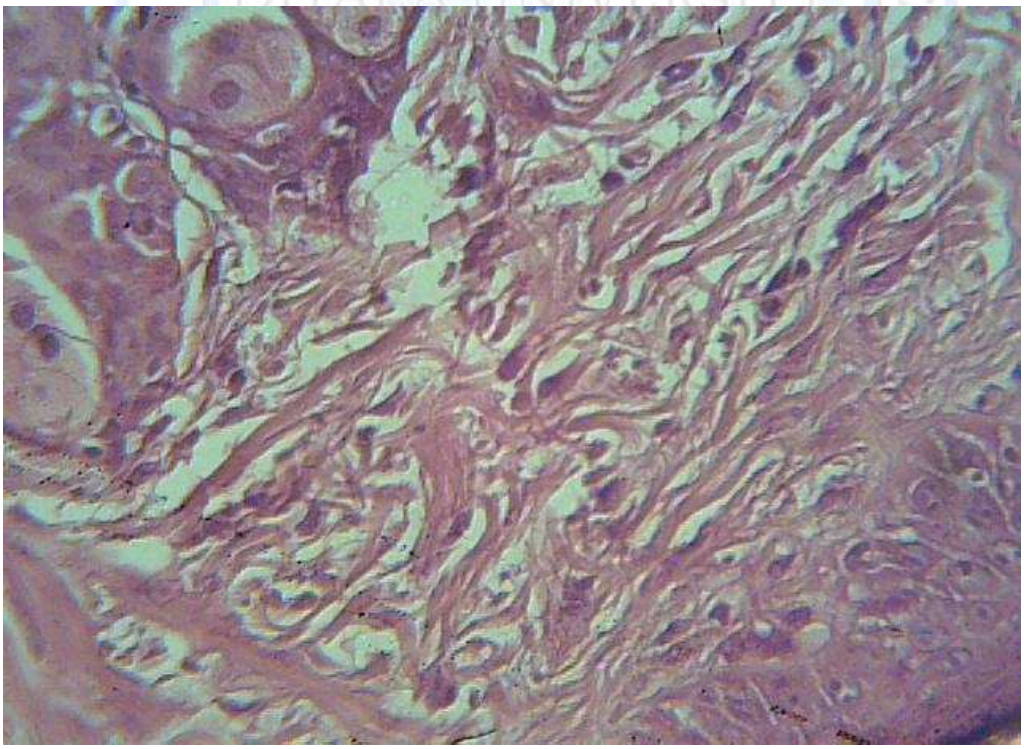

Figure (7): Histological section in skin of an animal infected with *S. aureus* show few mononuclear cells infiltration by H&E (400 X).

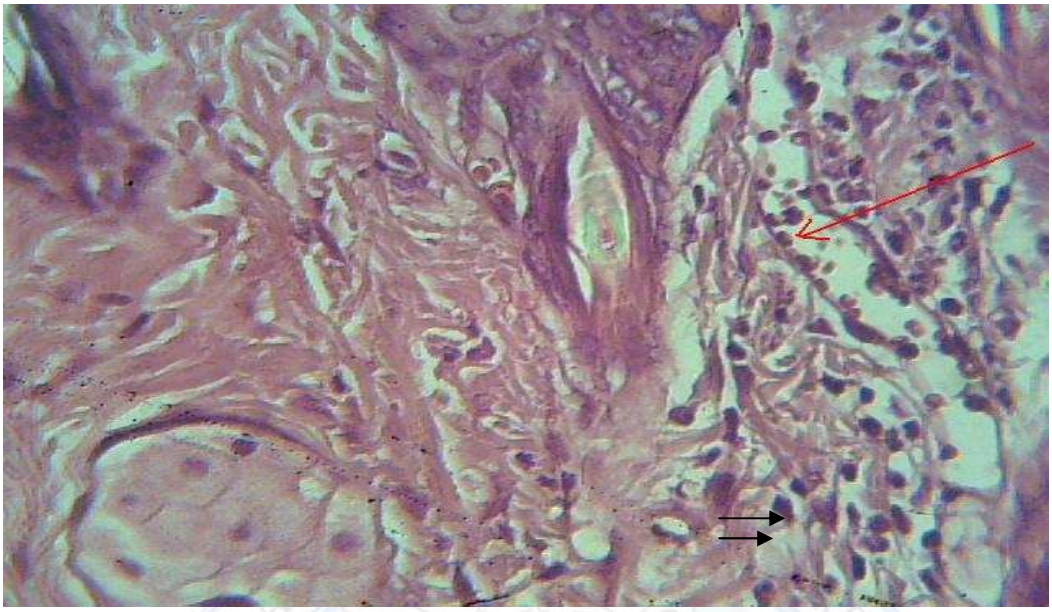

Figure (8): Histological section in skin of an animal infected with *P. aeruginosa* show congested blood vessels with inflammatory cells in the lumen (arrow) and inflammatory cells infiltration in the dermis and subcutaneous tissue (⇉) by H&E (400 X).

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