

## The pathogenic effects of the *Bacillus circulans* on some blood physiologic parameters and the histological changes of albino rabbits

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### Abstract

The study aimed to investigate the Pathogenic effects of *Bacillus circulans* and identification of some diagnostic features of this bacteria, The results roved that there were not any sickness state in the albino rabbits which treated with the inoculate of the bacteria *B.circulans*. There was no effects on some of hysiological parameters of the blood in the treated animals, also did not record any pathogenic effects in the liver and kidney tissues of the treated animals .

### Introduction

The genus *Bacillus* belongs to the order Eubacteriales and from suborder Eubacterrineae which includes the family Bacillaceae which forming endospores which generally is characterized by their ability to live earobicly and anearobicly and anearobicly and citrate reducing in addition to its secretion of catalase and glucose fermenting(Holt et al.,1994).The bacteria had great importance due to its quick growth and reproduction and its ability to breakdown various types of natural material that found in the nature(AL-Muslih and AL-Haidary,1984) for example ,the genus *Bacillus* include strains compose an important part of industry specially in enzymes production in addition to its role in microbiology field, these strains were developed through modern effective stratigies, most important of there genetic engineering techniques that its abilities increased in fast growth through fermenting cycles in short periods and increase in protein production, and that facilitate its usage in pharmacological and nutritional industrial field that most of their species have no pathogenic abilities (Schallmey et al.,2004).But this similarity between this bacteria and numbers of species that belonged to the same genus that have medical importance such as *B.cereus* which cause the food poisoning ,and *B.anthraxis* that responsible for anthrax made people afraid from its effects on the health of human and animal (Swedeues,1994).So this study aimed to investigate, some of diagnostic characteristics of *B.circulans* , study the pathogenic effects of this bacteria on albino rabbits and this include,

The physiological effect including (Erythrocyte ,Sedmentation Rate ESR ,white blood cell count ,packed cell volume (P.C.V.) ,hemoglobin concentration(Hb). The histological effects including study of histological effects in liver and kidney tissues.

### Materials and methods

1-The microorganisms that used in the study ,bacterial isolates of *B.circulans* obtained from advanced microbiology Lab./dep.of Biology/ College of science /University of Kufa .

2- lab .animals : Neuzeland albino rabbits were used in the study about 9 animals (683.5-206.1)g in weight

brought from the college of Medicin /Babil University .

### 3- culture media:

**A-Nutrient agar :**prepared by solving (23)g from the media 1L D.W. ,used in studying cultural features of the bacteria .

**B- Nutrient broth:**  
Prepared by solving (20)g of the media in 1L.D.W.used to activate growth of bacterial isolates .

**C-Blood agar:** prepared by solving (40)g of media in 1L.D.W.,used to investegate the ability of bacteria to analyse the blood (Collee et al.,1996).

**4- Confirmative diagnostic tests for *B.circulans*** study the presented features under examine the presented features of *B.circulans* colonies, and determine the size of colony and its color and study its consistence and purifying the colonies which have the characteristics of *B.circulans* on a dishes contain nutrient agar media.

### 5-Studying the microbiological features of *B.circulans* cells

A sample from the colonies that thought to be a *B .circulans* colony were tested and stained with Gram stain after fixed on a slide and examine the shape of bacteria cell ,and position of spores in it .

**1- Oxidase test** few drops of prepared oxidase indicator were put on filter paper then a small amount of the colony were taken with platine wire (string ) and put on the filter paper, if the colony colored with violet color after (10) seconds is a indictor of positive result (Macfaddin ,2000).

**2- Catalase test** a small amount of the bacteria isolate (of 24 hr.age) were put with a loop in a clean slide and then mixed with few drops of H<sub>2</sub>O<sub>2</sub> solution (30% concentration ),the forming of aerial babbles on the slide indicate the positive results(Macfaddin ,2000).

**3- Blood hemolysis test** the solid blood media was inoculated with the bacteria then incubated in 37C° for 24 hr. ,the green regions that appear around the colony means that the hemolysis is  $\alpha$  hemolysis (a partial hemolysis ) ,while the appearance of translosant regions around the growing colony means that the type of hemolysis is ( $\beta$  hemolysis )(a complete hemolysis) (Collee et al.

**4- Citrate Utilization :**citrate media was streaked with the ,2000). bacteria and incubate in 37 C° for 24 hr. and the result was positive by the converting of the color from green to blue (Collee et al. ,1996).

**5- Motility Test :** the tubes containing the motility media was innoculate by (stabbing ) with the bacteria ,then the tubes incubated under 37C° for 24hr.,the bacteria growth spread out of stab edges is an indicator of the ability of bacteria of

**6- Fermentation Test :**various sugars media motility ( Collee et al.,1996). tubes were inoculated by the bacterial isolate ,and incubated under 37C° for 24 hr., the result considered positive when the color of media changen from red to yellow and that is indicator of sugar fermentation and acid forming ,while the positive results of gas production indicated by forming gas babbles in durham tubes (Macfaddin,2000).

**7- Lecithinase test :**(100) ml of nutrient media was prepared according the manu factering company and sterilized in the autoclave under 121 C° and 1(atm.) for 20 mint.(Collee 1996),after the media cooled in water bath to (45C°) 5 ml of

yolk of was taken from sterilized shell egg by ethyl alcohol ,was taken by sterilized neddle and put in the cultural media the contents were shaken well then poured in petry dishes and left to cool in 37C° for 24 hr ., in the positive test a clear region formed in the media (Macfaddin ,2000) .

#### **Examination of the pathogenic effects in albino Rabbits**

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animals were prepared and grouped in 3 groups and treated as the following

**A-** first group include 3 animals injected with *B.circulans* innoculat after the bacteria were grown on nutrient broth and incubated for 24 hr., each animal injected with (1 ml) of the bacteria innoculated media per (1 kg) of animal weight .

**B-Second group :**compose of 3 animals injected with nutrient broth ,each animal injected with (1 ml) of the non innoculated media per kg of animal weight

**C- Third group :**composed of 3 animals injected with normal saline (1 ml /kg) of animal weights and then the result of experiment was observed and evaluated :

1-Clinical symptoms :The clinical symptoms and signs that appeared on experimental animals during two weeks were observed .

2- blood physiological tests :

**A-** total white blood cells count (5 ml ) of Turk solution were put in clean test tube and added to it (0.02 ml) of blood brought by sahly pipate ,the mixture shaken well then a drop from the mixture were put on slide champer covered with slid cover, left 2 minutes to stable ,then examined under the microscope (40 x power ) the W.B.C. counted in the four large squares in the corners (Brown ,1976) ,the total count obtained by using the following equation:  $W.B.C/ mm^3 = \text{number of counted cells} \times 50$  .

**B-** total hemoglobin concentration measure: (5 ml) of Drabkin solution put in a test tube and (0.02 ml) of blood added to it ,the tube shaken then left (10 min.) ,then the instrument were standardized then the tube were put in the instrument and the result read in 540 nanometer .(sood ,1992) .

**C-** Erythrocyte Sedimentation Rate (E.S.R) : (0.5ml) of sodium citrate (dilution solution ) in test tube and (2ml ) of the blood added ,mixed well ,and the mixture were taken and put in wistecreen pipete and suspended ventrically for (1hr.) then E.S.R. value were read in limit between the plasma and R.B.C , the results recorded (ml/hr.) (Brown ,1976) .

**D-** Packed Cell Volum P.C.V. : using the capillary tube ,the blood put in a capillary tubes and left (15 s) empty then one of two ends were closed by (industrial clay ) ,the put in high power centrifuge at (1000 R.P.M.) for five minute , the percentage of P.C.V.were recorded by using hematocrite ruler .

3- Histological diagnosis : The histological sections prepared from removed organs (Kidneys and livers ) in Dr.Asaad Al-Janabi lab .according to (sterens ,Bancyoft ,1982) .

**Statistica analysis :**

The experiment designed statically by using complete random design (C.R.D.) and the results analyzed by using least significant difference ,under propability level (0.05) (AL-Rawi and Khalaf Allah ,1980) .

## Results and Discussion

The confirmative diagnosis of *B.circulans*, the cultural description of *B.circulans* colony : the colonies of *B.circulans* which grown on nutrient agar appear as big smooth soft spherical colonies with spherical edga and brownish white tend to dark straw color proceeding with the colony age (after 48-72hr.) ,the diameter of the colonies were between (2-5mm) and that corresponded with (Collee et al.,1996;and Macfaddin ,2000).

Microscopical description of *B.circulans* : the microscopic examination of the fixed slide which stained with Gram stain of *B.circulans* bacteria showed that this bacterial G<sup>+</sup> bacilli ,had oval central spore ,they are single bacilli accumulate rarely as chains ,and these characteristics corresponded with Collee et al., 1996; and Macfaddin ,2000 .

### Biochemical tests :

The following are the results of biochemical test for this isolate:

Table

1:The results of biochemical test for this isolate.

	Tests	Results
1	Catalase	+
2	Oxidase	variable
3	Hemolysis	Hemolysis
4	Lecituinase	Hemolysis
5	sporelocayion	Hemolysis
6	Spore shape	central
7	Arabinose	-
8	Glucose	+
9	Maltose	+
10	Sucrose	+
11	Xylose	-
12	Mannitol	-
13	Mannose	-
14	Sorbitol	-
15	Citrate	+
16	motility	+

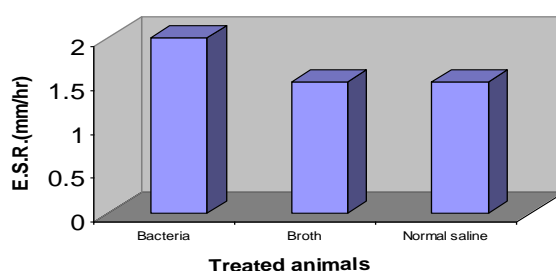
### The symptoms and clinical indications in the rabbits.

Table (2):The symptoms and clinical indications in the Rabbits.

Viewed observation on Rabbits			Period after injection(day)
Temperature	Feeding	Motility	
Normal	Normal	Normal	2
Normal	Normal	Normal	3
Normal	Normal	Normal	4
Normal	Normal	Normal	5
Normal	Normal	Normal	6
Normal	Normal	Normal	7

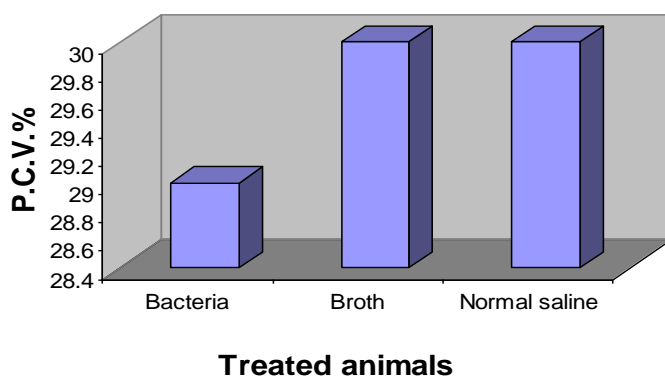
### The pathogenic effect in some physiological parameters of the blood .

A- E.S.R. The results showed that there wasn't significant increase in E.S.R. ,which was (2mm/hr.) and it was normal in comparison with the control which was (1.5mm/hr.) (figure 1) and this may due to that this bacteria lack pathogenic ability ,and it can't cause bacterial infection within the body ( Berkow& Chir ,1995) ,that the E.S.R. increasing primarily in the bacterial infection and chronic diseases (Berkow &Chir,1987) .



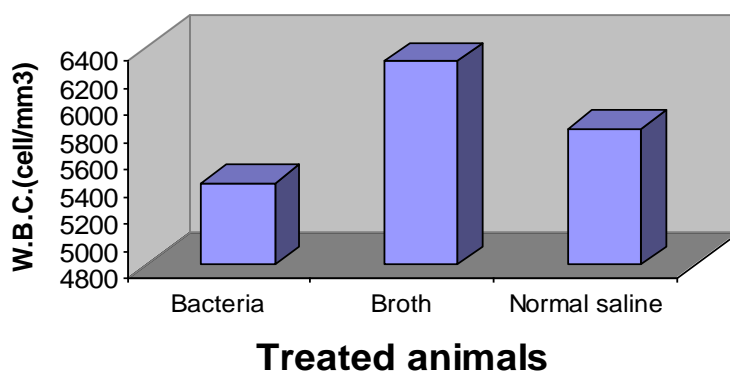
Figure(1):Effect of *B.circulans* in E.S.R.(mm/hr) in the albino Rabbits after aweek from injection.

B-P.C.V. The results (figure 2 ) showed that there was no significant differences in P.C.V. which was ( 29%) in comparison with the control group that was (30%) ,these results are within the normal values of P.C.V. in albino rabbit blood .



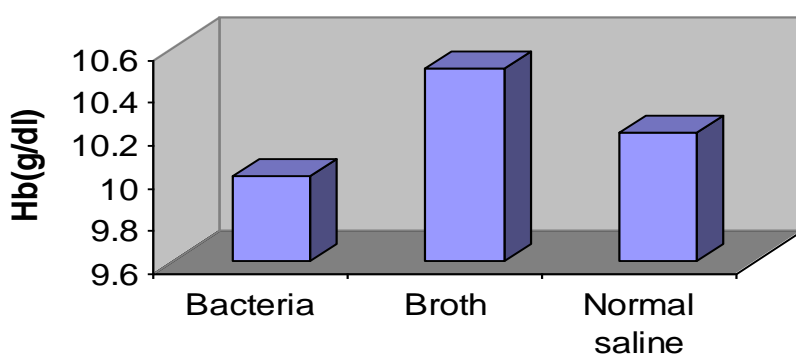
Figur(2):Effect of *B.circulans* in albino Rabbits

C-Total W.B.C. count : the results showed that there was no significant differences in W.B.C. count in the blood of *B.circulans* treated rabbits in comparison with the control , this indicate that this bacteria is not pathogenic because any changes didn't occur in total W.B.C. count indicating that there was no inflammation in various of body tissues like liver and kidney (Barbara ,1995).



Figure(3):Effect of *B.circulans* in total W.B.C.count after a week of injection in albino Rabbits

significant differences in Hb concentration the *B.circulans* treated rabbits when compared with the control group (figure 4) .



Figure(4):Effect of *B.circulans* in Hemoglobin cocentration in the albino Rabbits after a week from injection

### Histological changes :

Histological examination of the tissues of the *B.circulans* treated rabbit showed that the tissues of liver and kidney (figure7,10) identical with those of the control group (figure5,8) and that gives a clear

indication that histologically this bacteria is not pathogenic ,that it lack the virulent agents to cause any pathogenic effects .

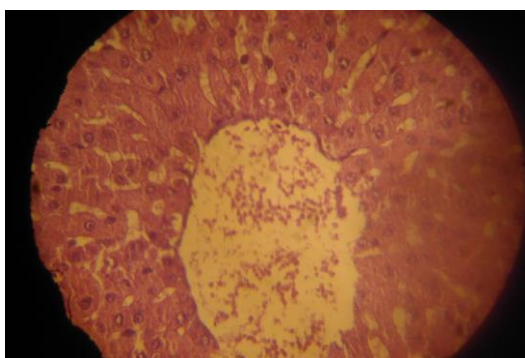


Figure (5) :Histological section of liver (1ml) from normal saline treated for one week(Haematoxlin, Eosin)(400X) .

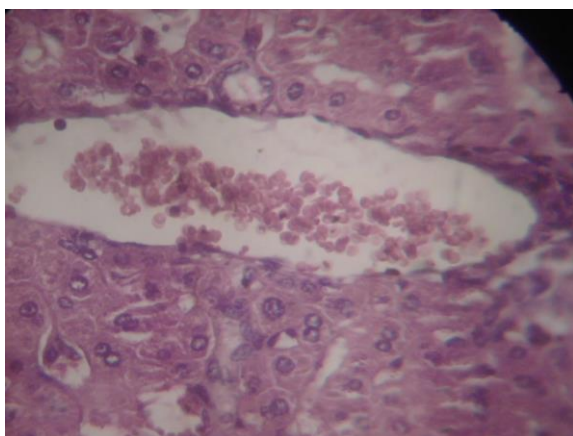


Figure (6) :Histological section of liver (1ml) from broth treated for one week(Haematoxylin,Eosin) (400X).

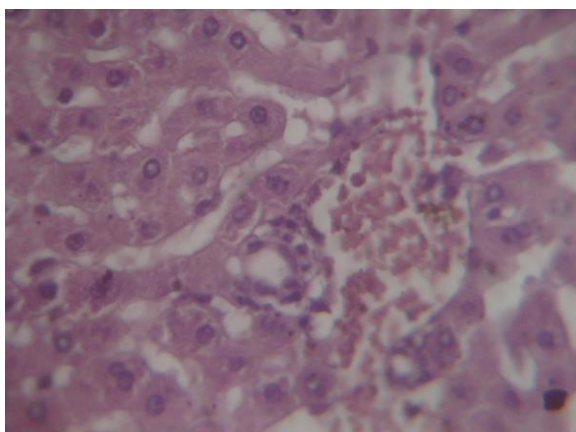


Figure (7) :Histological section of liver (1ml) from bacterial suspension( *B.circulans* ) treated for one week(Haematoxylin,Eosin) (400X).

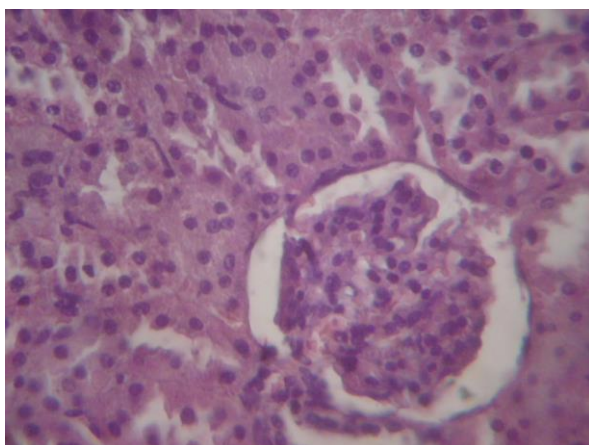
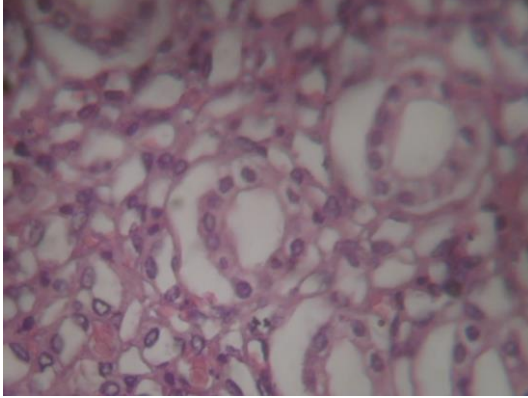


Figure (8) :Histological section of kidney (1ml) from normal saline treated for one week(Haematoxylin ,Eosin) (400X).





Figure (9) :Histological section of kidney (1ml) from broth treated for one week(Haematoxlin, Eosin) (400X).



Figure(10) :Histological section of kidney (1ml) from bacterial suspension ( *B.circulans* ) treated for one week(Haematoxylin ,Eosin) (400X).

### التأثيرات المرضية لبكتريا *B.circulans* على بعض معايير الدم الفسلجية والتغيرات النسيجية على الأرانب

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#### الخلاصة:

هدفت هذه الدراسة التحري عن التأثيرات المرضية للبكتريا *Bacillus circulans* والتعرف على بعض الصفات التشخيصية لهذه البكتريا .  
برهنت النتائج على عدم حدوث حالات مرضية لدى الأرانب البيض المجرعة ببلقاح البكتريا *Bacillus circulans* حيث لم تظهر تأثيرات معنوية في بعض معايير الدم الفسلجية للحيوانات المجرعة . كما لم تسجل أي تأثيرات مرضية في أنسجة أعضاء الكبد والكلى للحيوانات المجرعة.

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