

**Bacteriology studies on *Bacillus thuringiensis***AK. Al-Ghamdi<sup>1</sup> and Hanan, M. Sobhy<sup>2</sup><sup>1</sup> Medical Laboratory Technology Departments, Faculty of Applied Medical Sciences, King Abdulaziz University, Saudi Arabia.<sup>2</sup>Head of Biochemistry, Toxicology and Food deficiency Department- Animal Health Research Institute, Dokki, Gizza.[aalghamdi@kau.edu.sa](mailto:aalghamdi@kau.edu.sa); [AK6060@hotmail.com](mailto:AK6060@hotmail.com)

**Abstract:** Dipel 2x (*Bacillus thuringiensis*) was used to detect its antibacterial effect against group of aerobic and anaerobic bacteria, including *Clostridium perfringens* type B, *Escherichia coli*, *Salmonella typhimurium*, *Actinomyces pyogenes* and *Staphylococcus aureus*. The agar gel diffusion technique as well as biochemical analysis were used to obtain the results. The tested biological substance were effective against some common bacteria (*E. coli* and *S. typhimurium*), but less effective against *C. perfringens*. Dipel 2x was effective against aerobic bacteria (*E. coli* and *S. typhimurium*) but less effective against anaerobic bacteria, including (*C. perfringens* type B). Satisfactory results were obtained by oral administration of Dipel 2x in doses of 50 mg/100 gm b.wt. for 7 successive days into mature infected rats, which produced a significant decrease in the serum level of total protein, AST, AP activity, creatinine, bilirubin and ALT. Rats orally administered with antibiotic (chloramphenicol and enrofloxacin in doses of 27 mg and 0.5 mg/100 gm b.wt.) after infection by *E. coli* showed significant increases in total protein, creatinine, bilirubin levels, ALT, and ALK phosphates in serum [Hanan, M. Sobhy and AK. Al-Ghamdi. **Bacteriology studies on *Bacillus thuringiensis***. *N Y Sci J* 2013;6(12):54-58]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 8

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**1. Introduction**

*Bacillus thuringiensis* is an aerobic, spore forming, Gram positive rod-shaped bacterium that produces plasmid encoded toxins with insecticidal activity (Doyel *et al.*, 1985). It is associated with a parasporal protein crystal that is synthesized in the bacterium during sporulation and is very toxic to Lepidoptera Larvae and observed to be haemolytic.

*B. thuringiensis* is known for its enteropathogenic properties, which are partly because of the production of a variety of Lauricidal crystal proteins (Thomas *et al.*, 2003). When ingested by susceptible insect larvae, these crystal proteins are divided in the insect gut and activated. They bind to specific receptors on the surface of the mid-gut epithelial cells, forming transmembrane pores and causing cell lyses, but they are harmless to mammals and most other non-target species (Margaret *et al.*, 2009).

In the present study, *B. thuringiensis* spores were used as a biological substance (Dipel 2x) to detect its antibacterial effect (if present), *in vitro* and in adult rats infected with virulent strain *E. coli*.

The objectives of this study are as follows:

- 1- To detect the *in vitro* antibacterial activity of *B. thuringiensis* at different concentrations.
- 2- To summarize the laboratory animals' pathogenicity and to evaluate the safety of *B. thuringiensis* var. *kurstaki* by following different

exposure routes. The effects and safety of formulated products containing *B. thuringiensis* var. *kurstaki* administered orally in the rats before and after bacterial challenge were also demonstrated.

- 3- To study the effect of *B. thuringiensis* var. *kurstaki* on serum biochemical analysis and compare it with different antibiotics.
- 4- To investigate the role of *B. thuringiensis* as a treatment substance.

**2. Materials and Methods****Biological substance**

Dipel 2x *B. thuringiensis* var. *kurstaki* (32,000 international units of potency/mg) was obtained from the chemical and agricultural products division. It is available in white powder containing parasporal crystal and spores of *B. thuringiensis* Berliner, in addition to an approved adjuvant.

**Drugs**

1. Chloramphenicol 20% was used in dose of 27 mg/100 gm b.wt. for 7 days.
2. Enrofloxacin 10% was used in dose of 5 mg/100 gm b.wt. for 7 days.

**Bacterial strains**

Bacterial strains of *E. coli*, *S. typhimurium*, *A. pyogenes* and *S. aureus* were obtained from different disease conditions of buffaloes. In addition, a strain of *C. perfringens* was used in the *in vitro* sensitivity test.

**Laboratory animals**

Forty-five mature albino rats were fed on ordinary rations and water ad libitum.

#### Pathogenicity test

Different routes of injection were used for detection of the pathogenicity and/or toxicity of *B. thuringiensis* var. *kurstaki* in different concentrations as follows:

Twenty rats were divided into two groups. The first group received  $4 \times 10^7$  spore/kg b.wt. orally and the second group received  $10^7$  colony forming unit of bacteria by I/V route according to Woodrow (2002) and Stoll (2004).

All the animals were kept under observation for the detection of mortality and clinical manifestation of food intake.

#### Bacterial clearance

Microbial clearance was determined by frequently collecting faeces from tested animals after dosing and assaying the material for the presence of the tested organisms.

Internal organs, including mesenteric lymph nodes, lung, brain, kidney, liver and spleen were excised. Blood samples were collected immediately after dosing and at various time intervals thereafter.

Organs and blood samples were homogenized, diluted, and plated onto nutrient agar plates to determine the presence of the bacterium (Scherwood, 1989; Hard, 1990).

#### Bacterial activity

The antibacterial activity of *B. thuringiensis* was detected by using the agar gel diffusion technique according to Feingold and Martin (1982) against different bacteria (*C. perfringens* type B, *E. coli*, *S. typhimurium*, *A. pyogenes* and *S. aureus*) in 1 ml inoculums containing  $10^5$  bacterial cells per plate.

The biological substance was diluted, prepared in saline, and used in concentrations of 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 mg/ml.

All inoculated plates were incubated aerobically at 37 °C, whereas *C. perfringens* type B were incubated anaerobically.

The most effective antibiotics against *E. coli* and *C. perfringens* type B were as follows: gentamicin, chloramphenicol, enrofloxacin, penicillin, amoxicillin, lincomycin, oxytetracycline, streptomycin, and ceftriaxone.

#### Bacterial strains

Well-identified strains of *E. coli* and *C. perfringens* type B were prepared as follows:

##### 1. Preparation of used aerobic bacteria

Tested strains were obtained from different diseased conditions in buffaloes, including *E. coli* and *S. typhimurium* isolated enteritis.

*A. pyogenes* and *S. aureus* were obtained from mastitic buffaloes.

All the strains were identified bacteriologically by culture on different isolating media, examined morphologically, and then characterized biochemically according to Colle *et al.* (1996). The tested strain was sub-cultured into nutrient broth and incubated at 37 °C for 24 hrs. The number of viable organisms per/ml was determined by the plate-count agar method (Finegold and Martin, 1982) and matched with a McFarland tube no. 2 ( $3 \times 10^8$  viable organisms); 0.5 ml was injected (s/c/rat), according to Beal *et al.* (1988).

##### 2. Preparation of *C. perfringens* type B

A freeze-dried lyophilized stock culture of *C. perfringens* was dissolved in saline, and the contents of each were inoculated into one tube of cooked meat media and incubated anaerobically in a Gaspak jar over night at 37 °C. The growth of organisms was detected by gas bubbles developing at the base of the medium (Martin, 1983). A loopful was taken from each tube and inoculated onto sheep blood agar containing 200 ug/ml neomycin sulphates and then incubated anaerobically for 24 hrs. at 37 °C according to Cruickshank *et al.* (1975). Cooked meat broth was centrifuged at 3000 r.p.m. for 15 min; the sediment was suspended in a volume of 0.5% formal saline until it showed a degree of turbidity equivalent to McFarland tube no. 2 ( $3 \times 10^8$  viable organisms) and then administered orally according to Baily and Scott (1990).

##### Experimental design

The effect of Dipel 2x (*B. thuringiensis*) in infected rats administered orally at 50 mg/100 gm b.wt. for 7 successive days on bacteria was determined by serum biochemical analysis and compared with the groups that were administered chloramphenicol and enrofloxacin at 27 and 0.5 mg/100 gm b.wt., respectively, for 7 successive days.

Twenty-five mature albino rats were divided into five equal groups as follows:

Group 1: Control negative fed on basal diet

Groups 2 and 3: Both groups were infected by pathogen strain of *E. coli* and *C. perfringens*, kept without treatment for 3 days, and then orally administered Dipel 2x at dose 50 mg/100 gm b.wt. for 7 successive days and fed on basal diet.

Group 4: Infected by pathogen strain of *E. coli*, and after 3 days treated by enrofloxacin at dose of 0.5 mg /100 gm b.wt. for 7 successive days and fed on basal diet.

Group 5: Infected by pathogen strain of *C. perfringens*, kept without treatment for 3 days, and then treated by chloramphenicol at dose of 27 mg/100 gm b.wt. for 7 successive days and fed on basal diet.

##### Effect of Dipel 2x on biochemical analysis

At the end of the experiment, individual blood samples were obtained from rats and left to clot. Sera were separated for biochemical study.

The activities of AST, ALT, and AP were determined according to **Reitman and Frankel (1957)** and **Roy (1970)**, while total protein, creatinine, serum bilirubin levels were estimated according to **Peters (1969)**, **Henny et al. (1985)** and **Walters (1970)**.

The results were analyzed statistically according to **Snedecor (1969)** in order to determine whether a dosage group was +ve or -ve.

### 3.Results

#### Antibacterial activity

The results of the antibacterial activity test of Dipel 2x are tabulated in Table 1. As shown in the table, clear zones of inhibition indicated that the tested biological substance was effective against some common bacteria (*E. coli* and *S. typhimurium*) but much less effective against *C. perfringens*. No effect was found against *S. aureus* and *A. pyogenes*.

Enrofloxacin and chloramphenicol showed effectiveness against *E. coli* as facultative anaerobic bacteria but to a lesser extent against *C. perfringens* as the strict anaerobic bacteria used in the experiment in the control +ve groups of infected animals.

#### Pathogenicity infected animals

Oral administration: Showed no infectivity or toxicity.

Intravenous injection: The number of viable (CFU) count recovered after I/V injection of *B.*

*thuringiensis* (Dipel 2x) peaked by day 8. However these numbers decreased significantly by day 50. No clinical signs or changes in pathogenicity were observed under P/M examination.

#### Antibacterial effect

Bacteriological examination was performed in infected rats with virulent strain of *E. coli* without treatment with enrofloxacin or administered the *B. thuringiensis* solutions. All infected rats were died within 2-5 days and the infective bacteria were isolated from blood and internal organs of all infected dead rats.

In addition, a group of rats was administered *B. thuringiensis* after *E. coli* either alone or with enrofloxacin. In the case of *C. perfringens*, no mortalities were recorded in the groups of rats that received the *B. thuringiensis* only or in combination with chloramphenicol. *C. perfringens* isolates were revealed in the intestines of all examined rats.

#### Biochemical analysis

Administration of Dipel 2x orally at dose 50 mg/100 gm b.wt. after the infection by *E. coli* and *C. perfringens* showed significant decreases in all tested parameters, except ALT level, when compared with the negative group.

Oral administration of chloramphenicol and enrofloxacin at doses of 27 and 0.5 mg/100 gm. b.wt., respectively, after infection by *E. coli* and *C. perfringens* showed significant increases in total protein, creatinine, bilirubin levels, as well as ALT and AP activities in serum.

**Table (1):** Antibacterial activity of Dipel 2x against bacteria

Conc. mg/ml	<i>E. coli</i>	<i>S. typhimurium</i>	<i>C. perfringens</i>	<i>S. aureus</i>	<i>A. pyogenes</i>
	Zone of inhibition (mm)				
0.4	0	0	0	0	0
0.8	14.33	0	0	0	0
1.6	17.0	15.2	0	0	0
3.2	19.2	17.7	0	0	0
6.4	21.0	19.8	0	0	0
12.8	23.0	21.2	13.5	0	0

**Table (2):** Effect of oral administration of Dipel 2x on serum biochemical constituents and enzymatic activity in infected rats, mean± S.E (n=5)

Group	Dose mg/100gm b.wt.	Total protein g/dl	Creatinine mg/dl	Bilirubin mg/dl	ALT IU/L	AST IU/L	AP IU/L
Control -ve	-	7.64 ±0.24	23.42 ±1.599	107.072 ± 2.4	80.26 ± 3.8	23.43 ±0.105	32.997 ±1.6
<i>E. coli</i> + Dipel 2x	50	7.04 ±0.17	24.55 ±1.2	120.42** ±1.4	180*** ± 10.94	23.32 ±0.79	30.47 ±0.42
<i>C. perfringens</i> + Dipel 2x	50	8.39* ±0.18	33.64*** ± 0.35	115.79** ±0.73	183.26*** ± 8.7	51.69*** ±1.9	68.86*** ±0.77
<i>E. coli</i> + Enrofloxacin	0.5	8.36* ±0.196	26.89 ±1.0	59.16*** ±1.5	196.5*** ± 3.95	50.58*** ±0.62	32.38 ±1.34
<i>C. perfringens</i> +Chloramphenicol	27	6.36** ±0.33	25.56 ±0.68	83.7*** ±4.2	86.23 ± 6.8	20.98*** ±0.69	65.45*** ±1.01

#### 4. Discussion

*B. thuringiensis* is a Gram positive bacilli, spore forming bacterium that produces plasmid-encoded toxins with insecticide activity (Doyel *et al.*, 1985).

The results of the antibacterial activity tests of Dipel 2x are shown in Table 1. The tested biological substances were effective against some common bacteria (*E. coli* and *S. typhimurium*), but much less effective against *C. perfringens*. Furthermore, the results showed that Dipel 2x was effective against some facultative anaerobic bacteria (*E. coli* and *S. typhimurium*) and to a lesser extent against strict anaerobic bacteria (*C. perfringens* type B).

No effect was found against *S. aureus*, *A. pyogenes*. These variations in the effect of *B. thuringiensis* on bacteria are in agreement with Som *et al.* (2006) and Hanan *et al.* (2000), who observed that *B. thuringiensis* has an effect on some bacteria that were found resistant to some antibiotics.

The results of the pathogenicity test in orally administered rats showed no infectivity and toxicity.

The number of viable bacterial count recovered after I/V injection of *B. thuringiensis* (Dipel 2x) peaked by day 8. However, these numbers decreased significantly by day 50, without any clinical signs or pathogenic changes under P/M examination. These findings agree with the results reported by Woodrow (2002), Stoll (2004) and Berg (2009), who proved no harmful effect of *B. thuringiensis* (Dipel 2x) when administered orally or by I/V route.

Bacteriological examination of infected rats with virulent strain of *E. coli* without treatment with enrofloxacin or administration of *B. thuringiensis* solution died within 2 to 5 days. The infective bacteria were isolated from the blood and internal organs of all infected dead rats.

In the case of *C. perfringens*, no mortalities were recorded in groups of rats that received *B. thuringiensis* only or chloramphenicol. In contrast, the infected rats kept without treatment died within 2 days post-infection. and *C. perfringens*, were isolated from the intestines of all dead rats, which is in agreement with Hanan *et al.* (2000).

The administration of Dipel 2x orally to infected rats at dose of 50 mg/100 gm b.wt. after infection by *E. coli* and *C. perfringens* showed significant decreases in all tested parameters, except ALT levels, when compared with the control negative group.

The oral administration of chloramphenicol and enrofloxacin at doses of 27 and 0.5 mg/100 gm b.wt., respectively, after the infection by *E. coli* and *C. perfringens* showed significant increases in total protein, creatinine, bilirubin level, ALT and AP activities in serum, these results agree with Hanan *et al.* (2000).

It was concluded that as an antibacterial agent, Dipel 2x has a detectable effect on some bacteria either *in vitro* or *in vivo*, which indicates its potential for use in the treatment of some animal bacterial diseases, either alone or with other antibiotics, such as enrofloxacin and /or chloramphenicol, which enhance its effect and overcome the side effects of antibiotics when used alone. Dipel 2x also can reduce the effect of antibiotics on biochemical changes in serum.

On other hand, it was found that active microbial of *B. thuringiensis* has the ability to cross the gastro intestinal tract barrier and appear systemically in tissue organ and blood (Thomas *et al.*, 2003). Hence, *B. thuringiensis* acts on the bacteria before secreting toxin.

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