

Clinico-Pathological Changes in Wistar Rats Administered *B. thuringiensis* Isolate from Soft Cheese 'Wara'

Victoria Olusola. Adetunji and Sarah Chinonyerem Anyanwu

Department of Veterinary, Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: This study evaluated the safety of bacteriocin producing *Bacillus thuringiensis* (*B. thuringiensis*) at 10^2 and 10^4 CFU in food preservation. This was assessed by testing the virulence and toxicity induced in rats due to administration of *B. thuringiensis* and its bacteriocin. Ten Wistar rats were randomly divided into five groups of two animals each having a male and female rat representative. The blood samples were collected for haematological analysis at day 0, 2, 10 and 15. The liver, kidney, testes and ovaries were examined for histopathological changes. There were no toxic changes related to the administration of *B. thuringiensis* in female rats at 10^2 and 10^4 CFU and in the male rat at 10^2 CFU. Male rat given *B. thuringiensis* at 10^4 CFU showed severe germinal erosion of the testis, while there was periportal hepatic necrosis of the liver and severe diffuse tubular necrosis of the kidney when its bacteriocin was administered at the same concentration. It can be deduced that female consumers are at less risk when compared to male consumers of products in which *B. thuringiensis* or its bacteriocin are being incorporated. Also, lower doses appear to be safer for effective use of this *Bacilli* organism and its bacteriocin.

Key words: Bacteriocin • *Bacillus thuringiensis* • Rats • Virulence • Toxicity

INTRODUCTION

In attempt to control pathogenic bacteria in food, the production of antimicrobial peptides from bacteria "bacteriocins" has been given consideration. Bacteriocins are compounds produced by bacteria that have a biologically active protein moiety and bactericidal action [1]. They are heterogeneous compounds which vary in molecular weight, biochemical properties, activity spectra and mechanism of action [2]. These polypeptide antibiotics can possess bactericidal, fungicidal, metal-chelating and immunomodulating activities. They are frequently found in secondary metabolites produced by various microorganisms, such as Gram-positives bacteria of the genus *Streptomyces*, lactic acid bacteria and genus *Bacillus* [3]. Some bacteriocins kill only bacteria belonging to the same species as producer whereas other bacteriocins kill a broad range of Gram positive bacteria [4, 5]. The incorporation of these compounds as biopreservative ingredient into model food has been shown to be effective in the control of pathogenic and spoilage micro-organisms [6].

Bacillus is an interesting genus to investigate since it produces a diverse array of antimicrobial peptides representing several different basic chemical structures [7]. Many species of *Bacillus* are of considerable importance example members of the genus *Bacillus* produce antibiotics like bacteriocin, gramicidin and polymyxin. Microbial insecticides *Bacillus thuringiensis* has been examined as an alternative method of pest control due to increased public concern of the potential adverse environmental effects associated with the heavy use of chemical pesticides.

B. thuringiensis is a ubiquitous Gram – positive soil bacterium and has toxicity against a wide spectrum of lepidopteran insect [8]. Some formulations can be used on essentially all food crops. In addition, *B. thuringiensis* is an insecticide with unusual properties that make it useful for pest control in certain situation. Several strains can infect and kill insects, because of this property. *B. thuringiensis* produces a parasporal inclusion body during sporulation usually referred to as a crystal is made of proteins and exerts its mode of action by dissolving in the alkaline environment of the host insect midgut causing lyses of the midgut epithelial cells membranes,

Corresponding Author: Victoria Olusola. Adetunji, Department of Veterinary,
Public Health and Preventive Medicine, University of Ibadan, Ibadan Nigeria.
Tel: +2347040979193, E-mail: vadetunji@gmail.com & vo.adetunji@mail.ui.edu.ng.

eventually leading to death of the larvae [9-11]. *B. thuringiensis* is considered safe to people and non target species, such as wildlife [12, 13].

This present study aimed at investigating the acute toxicity level of *Bacillus thuringiensis* and its bacteriocin in Wister albino rats to ascertain the safety during its use.

MATERIALS AND METHODS

Bacterial Strain: *B. thuringiensis* was harvested from soft cheese (wara) and identified on the basis of its cultural, morphological, physiological and biochemical characteristics [14]. The isolate was examined for bacteriocin production via inhibition zone using the Agar Well Diffusion (AWD) assay [15]. This organism had shown inhibition against *Listeria monocytogenes*, *Staphylococcus aureus* and *Micrococcus leutus*.

Test Animals: Male and female albino rats, *Rattus norvegicus albinos*, aged 9-10 weeks old weighing 60-90g were purchased from the animals house, University of Ibadan, Ibadan, Nigeria. Rats were kept under the laboratory conditions of 25±5°C and 65±5% R.H, three weeks to stabilize. They were housed in metal cages (25×20×15cm) and maintained on mouse cubes (protein: 21% min, fat: 3.5% min, fibre: 6.0% max, calcium: 0.8%, o.8) from Ladokun feeds Ltd. Ibadan and also on water. This diet contained all the dietary needs and was obtained from Mokola market, Ibadan, Oyo State, Nigeria. Animal experiments and housing procedures were performed in accordance to the animal care rules and they were approved by the authorities of the University.

Preparation of Inoculum: *B. thuringiensis* from soft cheese was stored in glycerol at -20°C and was purified by sub culturing of the colonies in plated media and incubated at 30 - 37°C for 24 hours. In an effort to obtain a pure strain, a discrete microbial colony was picked with the aid of a sterile wire loop. This was streaked out on a new nutrient agar plate and incubated at 37°C. The sub-culturing was done three times to obtain a pure colony. The concentration of *B. thuringiensis* and its bacteriocin that was inoculated into the rats were determined through a serial dilution at 10⁻¹⁰. 0.1ml of the sample strain was inoculated into a Petri dish containing prepared nutrient agar and incubated at 30-37°C for 24 hours which gave a total Colony Forming Unit (CFU) of 5×10¹⁴ CFU/ml while at 10²CFU and 10⁴CFU gave 0.1ml and 0.3ml respectively.

Detection of antimicrobial activity by Agar Well Diffusion assay (AWD):

Nutrient agar plates seeded with *Micrococcus luteus* was used for the agar well diffusion test. 5mm diameter wells were created with a sterile club. The wells were then layered with viscous nutrient agar. 10²cfu/ml of *B. thuringiensis* was placed in wells and incubated at 37°C for 24hours, the diameter of the zone of growth inhibition were then measured in mm.

Harvesting of Bacteriocin:

To test for the antimicrobial activity of the bacteriocin produced by *B. thuringiensis*. *B. thuringiensis* was grown in 10mls of nutrient broth and incubated at 37°C for 18-24hours. The broth was centrifuged at 3500 revolution for 15-20 minutes after which the bacteriocin was drawn out into another test tube using a pipette to prevent mixing with the bacteria cells below the test tubes. The supernatant was decanted into sterile test tubes, adjusted to pH 6.5 - 7.0 with NaOH (40G/1000 ml) to remove organic acid effect. H₂O₂ was neutralized by addition of catalase from bovine liver at 200 µ/ml. The mixture of the supernatant of culture, NaOH and catalase was filtered and sterilized with a 0.2 µm Millipore filter membrane. Inhibitory effect of free bacteriocin on test bacteria was then determined by agar well diffusion method using the filtrate from the mixture and the supernatant alone.

Clinico-Pathological Study

Experimental Design: Ten rats were divided randomly to five groups each of two rats. Each group has a male and female with their replicates. At day 0, before the inoculation of *B. thuringiensis* and its bacteriocin, the rats were weighed and blood samples were collected which served as baseline data. The first and second groups received intramuscular doses of *B. thuringiensis* at quantities equivalent to 10²CFU and 10⁴CFU, respectively. The third and fourth groups received intramuscular doses of bacteriocin at quantities equivalent to 10²CFU and 10⁴CFU, respectively.. The control groups were given distilled water. At day 2 and 10 post inoculums, blood sample were collected and on day 15, the rats were re-weighed and blood samples were collected after which the rats were sacrificed immediately.

Hematological Examination: One ml of blood sample was collected from each rat in replicate. This was done by inserting a capillary tube into the media canthus of the eye and blood was drawn which flowed through the capillary tube into an Eppendoff tube containing EDTA (50ul/ml) for hematological analysis.

The hemoglobin concentration was done as described by Schalm *et al.* [16] using the cyanomethemoglobin method. Packed cell volume (PCV) was done by conventional method of filling the capillary tube with blood as described by Schalm *et al.* [16] and read with a microhematocrit reader. Erythrocyte count was determined by the hemocytometer method as described by Coles [17]. Total leucocytes and leucocytes differential count were also determined. Erythrocyte indices were determined from values obtained from red blood cell count, hemoglobin concentration and packed cell volume.

Histopathological Study: The influence of *B. thuringiensis* and its bacteriocin on the histopathology of the liver, kidney and reproductive organs were investigated at the end of this experiment. The animals were sacrificed with use of cervical dislocation. A ventral midline incision was made with scalpel blade from the xyphoid cartilage to the pelvic area. Also the ribs were crushed to expose the thoracic cavity. The internal organs were appropriately exposed and the liver, kidney and reproductive organs were harvested and fixed in 10% neutral formalin in labeled bottles and then prepared for histopathological examination according to Lillie *et al.* [18].

Statistical Analysis: Statistical analysis of hematological parameters were carried out by Analysis of variance (ANOVA) at a level of significance of $P < 0.05$ between the baseline data and subsequent days. The results were quoted as means \pm SEM.

RESULTS

Clinical Findings: Three hours after the collection of blood samples on the 10th day, two mortalities were recorded in male rats given *B. thuringiensis* and its bacteriocin at 10⁴CFU.

Hematological Findings: The haematological results in the female rats treated with *B. thuringiensis* showed no significant changes in PCV at 10²CFU at day 2, 10 and 15 but it significantly decreased ($P < 0.05$) when 10⁴CFU was given at day 2 as compared to the control. There was a significant increase in RBC count at day 15 and day 10, 15 at 10²cfu and 10⁴CFU, respectively. No significant change was observed in the Hb at 10² and 10⁴CFU throughout the experimental period except a significant increase at day 2 when 10²CFU was given. At day 2, there was a significant decrease ($P < 0.05$) of WBC and lymphocyte count at 10⁴CFU. At 10² and 10⁴CFU MCH significant increase ($P < 0.05$) in MCH at day 10 and 15 while it decreased significantly at day 10 when 10⁴CFU was administered. Also MCV of female rat at 10⁴CFU significantly increased at day 10 (Table 2).

Female rats administered 10²CFU of the bacteriocin of *Bacillus thuringiensis* at day 2 and 15 produced a significant decrease in PCV. The male rat showed no significant change in RBC count in the different doses throughout the experimental period as compared to the control that showed a significant increase in RBC count at day 15. Female rat induced with 10²CFU of *Bacillus thuringiensis* bacteriocin produced a significant decrease

Table 1: Weights Of Rat Before And After Inoculation Of *Bacillus Thuringiensis*(A) And Its Bacteriocin

	FOA ²	MOA ²	FOA ⁴	MOA ⁴	FbA ²	MbA ²	FbA ⁴	MbA ⁴	FCO	MCO	FCb	MCS
Before	80	80	80	60	90	80	70	60	80	60	80	80
After	80	80	75	-	80	80	70	-	80	59	80	80

FOA²: female rat given Bacillus organism at 10² CFU

MOA²: male rat given Bacillus organism at 10² CFU

FOA⁴: female rat given Bacillus organism at 10⁴CFU

MOA⁴: male rat given Bacillus organism at 10⁴CFU

FbA²: female rat given bacteriocin at 10² CFU

MbA²: male rat given bacteriocin at 10² CFU

FbA⁴: female rat given bacteriocin at 10⁴CFU

MbA⁴: male rat given bacteriocin at 10⁴CFU

FCO: female rat used as control for the Bacillus organism

MCO: male rat used as control for the Bacillus organism

FCb: female rat used as control for the bacteriocin

MCb: male rat used as control for the bacteriocin

Table 2: Hematological data of female rats before (Baseline) and after administration of *B. thuringiensis*

Organism	Type	Dosage		PCV	Hb (g/dl)	RBC (x10 ¹² /μl)	WBC (x10 ⁹ /μl)	PLT (x10 ⁹ /μl)	MCV	MCH	LYM	NEUT
Female	A	10 ²	Baseline	39.50±1.50	13.10±0.40	12.35±0.01	23.40±5.80	15.00±1.00	31.50±1.50	10.00±0.00	70.00±0.00	30.00±0.00
			Day 2	27.50±0.50	9.05±0.15*	7.77±0.43	11.50±0.90	11.00±1.00	35.00±1.00	11.50±0.50	33.00±3.00	67.00±3.00
			Day 10	48.50±1.50	16.10±0.60	24.34±1.50	23.50±1.10	9.00±1.00	19.50±0.50	65.50±1.50*	71.00±1.00	29.00±1.00
		Day 15	35.00±2.00	11.55±0.65	14.53±0.15*	10.10±0.50	11.00±1.00	23.50±1.50	79.00±4.00*	64.00±1.00	34.50±0.50	
		10 ⁴	Baseline	39.00±0.00	12.90±0.00	12.41±0.01	29.00±0.60	9.00±1.00	31.00±0.00	60.50±0.50	39.50±0.50	
			Day 2	26.50±0.50*	8.90±0.00	6.13±1.07	8.80±1.60*	12.00±2.00	44.00±8.00	14.50±2.50	27.00±1.00*	68.00±4.00
	Day 10		46.50±1.50	15.40±0.50	27.18±1.06*	21.40±1.20	10.50±1.50	16.50±0.50*	56.50±0.50*	64.00±1.00	35.00±1.00	
	Control	Control	Baseline	37.00±3.00	12.20±1.00	20.72±0.44*	12.30±1.30	17.50±2.50	17.50±1.50	58.50±6.50	57.00±1.00	43.00±1.00
			Day 2	37.00±3.00	12.20±1.00	20.72±0.44*	12.30±1.30	17.50±2.50	17.50±1.50	58.50±6.50	57.00±1.00	43.00±1.00
			Day 15	41.00±2.00	13.55±0.65	15.02±0.58	16.20±1.40	10.50±1.50	27.00±2.00	95.00±3.00*	63.00±7.00	37.00±7.00

*Mean is significantly different from baseline value at $P < 0.05$.

*Each value represents mean \pm SD PCV-Packed cell volume; Hb-haemoglobin; RBC, red blood cell count; WBC, White blood cells; PLT, Platelet count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; LYM, lymphocyte; NEUT; neutrophil A, *Bacillus thuringiensis*, 10², 10²CFU; 10⁴, 10⁴CFU.

Table 3. Hematological data of female rats before (Baseline) and after bacteriocins of *B. thuringiensis* application.

Type	Dosage		PCV	Hb (g/dl)	RBC (x10 ¹² /μl)	WBC (x10 ⁹ /μl)	PLT (x10 ⁹ /μl)	MCV	MCH	LYM	NEUT		
Bacteriocin Female	A	10 ²	Baseline	37.50±1.66	12.38±0.56	10.00±0.61	16.35±1.63	13.75±1.03	37.25±1.60	12.00±0.58	75.25±2.21	25.00±2.38	
			Day 2	32.50±1.19*	10.75±0.42*	14.31±3.07	20.75±5.23	11.00±0.58*	26.50±6.54	8.50±2.18	31.25±8.77*	65.75±6.91*	
			Day 10	43.75±1.65	14.50±0.56*	25.20±1.49*	16.55±2.89	9.75±1.44	17.25±0.48*	57.25±1.75*	66.25±4.21	33.50±4.35	
		Day 15	36.25±2.17*	11.95±0.73	17.93±2.22*	9.63±1.50	9.25±1.25	20.00±1.22*	67.75±4.50*	62.25±4.48	36.50±4.63		
		10 ⁴	Baseline	45.00±0.00	14.90±0.00	12.64±0.00	30.20±0.00	14.00±0.00	30.00±0.00	30.00±0.00	70.00±0.00	30.00±0.00	
			Day 2	26.50±0.50	8.70±0.20	7.20±1.06	10.00±2.40	13.50±1.50	37.00±5.00	12.50±1.50	31.50±1.50	68.50±3.50	
	Day 10		44.00±0.00	14.70±0.00	27.21±0.01	8.45±0.25	12.00±0.00	16.00±0.00	54.00±0.00	57.50±0.50	41.00±1.00		
	Control	Control	Baseline	36.50±2.50	12.05±0.85	11.04±1.36	21.20±9.40	9.00±0.00	33.00±2.00	10.50±0.50	80.00±0.00	20.00±0.00	
				Day 2	27.00±0.00	8.90±0.00	5.16±0.00	9.30±0.10	19.00±1.00	52.00±0.00	17.00±0.00*	37.50±2.50*	62.50±2.50*
				Day 10	46.00±0.00	15.20±0.00	25.24±0.00	18.80±0.00	15.00±0.00	18.00±0.00	60.00±0.00	76.00±0.00	24.00±0.00
			Day 15	36.00±0.00	10.40±0.00	18.10±0.00	12.00±0.00	8.00±0.00	24.00±0.00	84.00±0.00	65.00±0.00	27.00±0.00	

*Mean is significantly different from baseline value at P<0.05.

*Each value represents mean ± SD

PCV-Packed cell volume; Hb-haemoglobin; RBC, red blood cell count; WBC, White blood cells; PLT-Platelet count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; LYM, lymphocyte; NEUT; neutrophil, A *Bacillus thuringiensis*; 10², 10²CFU; 10⁴, 10⁴CFU.

Table 4: Hematological data of male rats before (Baseline) and after *B. thuringiensis* application

Organism	Type	Dosage	PCV	Hb (g/dl)	RBC (x10 ¹² /μl)	WBC (x10 ⁹ /μl)	PLT (x10 ⁹ /μl)	MCV	MCH	LYM	NEUT		
Organism	A	10 ²	Baseline	35.50±2.50	11.80±0.90	10.95±1.31	20.10±8.70	13.50±1.50	32.50±1.50	10.50±0.50	56.50±0.50	43.50±0.50	
			Day 10	41.00±1.00	13.55±0.35	22.44±0.20	17.00±0.20	10.50±0.50	36.00±0.00	18.00±4.00	30.00±0.00*	65.00±5.00	
			Day 15	37.00±1.00	12.30±0.40	16.26±0.18	8.80±0.20	15.00±1.00	18.00±1.00	60.00±1.00*	73.00±1.00*	26.50±1.50*	
		10 ⁴	Baseline	37.50±2.50	12.45±0.75	11.02±1.22	14.60±2.20	9.50±0.50	22.50±0.50	76.00±2.00*	60.00±0.00	38.50±0.50	
			Day 2	26.00±1.00	8.35±0.15	8.41±1.01	11.60±0.20	9.00±1.00	33.50±1.50	10.50±0.50	71.50±0.50	28.50±0.50	
			Day 10	37.50±1.50	12.40±0.50	19.18±0.98	12.80±2.60	12.50±2.50	43.50±8.50	10.00±1.00	16.50±8.50	78.50±3.50*	
	Control	Control	Baseline	26.50±0.50	8.80±0.10	9.92±0.32	9.60±0.00	13.50±1.50	19.00±0.00	64.50±0.50*	55.00±1.00*	44.00±1.00*	
				Day 2	28.50±0.50	9.45±0.25	6.08±0.18*	7.40±2.60	13.00±2.00	26.50±0.50	8.50±0.50	64.50±0.50	35.50±0.50
				Day 10	41.00±0.00	13.70±0.00	20.76±0.00	18.20±0.00	20.00±0.00	46.50±0.50	15.00±0.00*	43.50±1.50	55.00±1.00*
			Day 15	38.50±2.50	12.80±0.90	17.24±0.36*	11.30±0.30	10.50±4.50	19.00±0.00	65.00±0.00	56.00±0.00	44.00±0.00	

*Mean is significantly different from baseline value at P<0.05.

*Each value represents mean ± SD

PCV-Packed cell volume; Hb-haemoglobin; RBC, red blood cell count; WBC, White blood cells; PLT-Platelet count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; LYM, lymphocyte; NEUT; neutrophil A, *Bacillus thuringiensis*; 10², 10²CFU; 10⁴, 10⁴CFU.

Table 5: Hematological data of male rats before (Baseline) and after administration of bacteriocins of *B. thuringiensis*

Type	Dosage	PCV	Hb (g/dl)	RBC (x10 ¹² /μl)	WBC (x10 ⁹ /μl)	PLT (x10 ⁹ /μl)	MCV	MCH	LYM	NEUT		
Bacteriocin Male	A	10 ⁴	Baseline	31.50±6.50	10.45±2.25	9.84±1.36*	23.70±5.50	11.00±1.00	30.00±1.00	9.50±0.50	69.00±3.00	31.00±3.00
			Day 2	24.50±0.50	8.05±0.15	4.66±1.38*	9.50±1.10	9.00±1.00	58.00±17.00	19.50±6.50	22.00±2.00	77.50±2.50
			Day 10	45.00±1.00	14.95±0.25	27.74±0.50	10.75±1.85	10.50±0.50	16.00±0.00*	56.00±3.00*	63.50±6.50	36.00±6.00
		Day 15	43.00±0.00	14.20±0.00	22.26±0.18	11.50±0.30	11.00±1.00	19.00±0.00	63.50±0.50	80.00±0.00	19.50±0.50	
		Control	Baseline	40.00±1.00	13.20±0.30	12.64±2.56	15.90±4.70	11.00±1.00	32.50±7.50	10.50±2.50	72.50±2.50	27.00±3.00
			Day 2	24.00±1.00	7.85±0.35*	4.34±0.26	6.20±1.00	14.00±2.00	55.50±5.50	18.00±2.00*	22.00±2.00	77.50±2.50
	Day 10		40.00±0.00	13.20±0.00	20.22±0.00	12.40±0.00	16.00±0.00	19.00±0.00	65.00±0.00	70.00±0.00	30.00±0.00	
	Day 15	32.00±0.00	10.50±0.00	13.68±0.40	18.55±0.75	12.00±2.00	23.00±1.00	76.50±2.50*	61.00±1.00	37.00±3.00		

*Mean is significantly different from baseline value at P<0.05.

*Each value represents mean ± SD

PCV-Packed cell volume; Hb-haemoglobin; RBC, red blood cell count; WBC, White blood cells; PLT-Platelet count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; LYM, lymphocyte; NEUT; neutrophil A, *Bacillus thuringiensis*; 10², 10²CFU; 10⁴, 10⁴CFU.

Histopathological findings

(P<0.05) of RBC count and Hb at day 2 while at day 10, both parameters significantly increased. At day 10 and 15, MCV decreased significantly while MCH significantly increased when 10²CFU of the bacteriocin was induced in the female rats. At day 2, there was a significant decrease in lymphocytes while neutrophil increased when 10² CFU of the *Bacilli* bacteriocin was administered in the female rat (Table 3).

At day 10 and 15 male rat given *B. thuringiensis* at 10²CFU showed a significant increased in MCH while when given at 10⁴CFU on day 10, it also showed a significant decrease. There was a significant

decrease of lymphocyte at day 2 and an increase on day 10 when 10² CFU of *B. thuringiensis* was administered into the male rat while at 10⁴CFU it increased significantly on day 10. At 10²CFU neutrophil showed a significant decrease at day 10 and at 10⁴CFU, it increased significantly at day 2 and 10 when administered into the male rats (Table 4).

Male rats treated with the bacteriocin of *B. thuringiensis* showed that at 10⁴CFU, PCV significantly decreased on day 2. There was a significant decrease of MCV and a significant increase in MCH at day 10 in the male rat (Table 5).

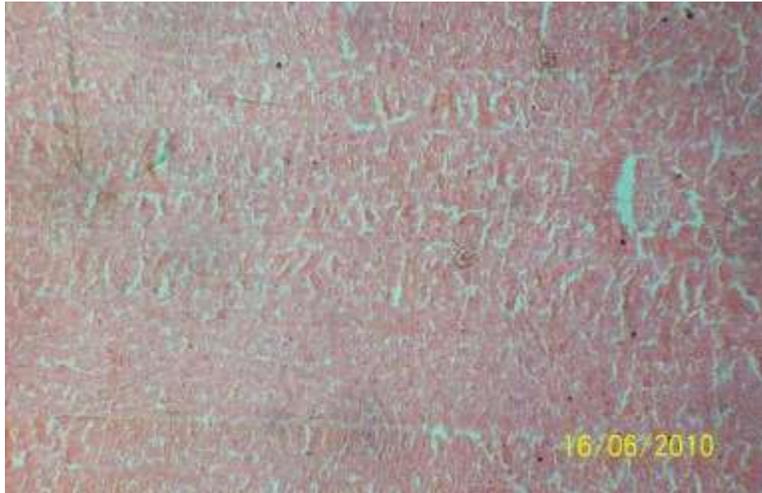


Fig. 1: Liver from male rat receiving 10^2 CFU *Bacillus thuringiensis*
Note: no visible lesion seen(H &E X 100)

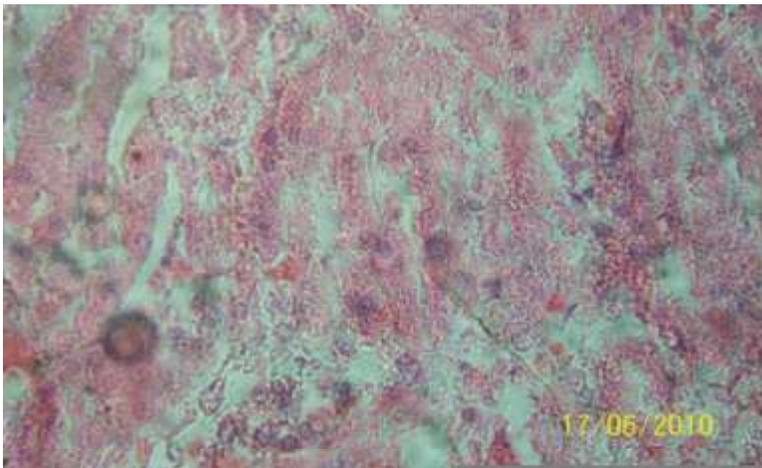


Fig. 2: Kidney from female rat receiving 10^4 CFU *Bacillus thuringiensis*
Note: no visible lesion seen (H & E x 400)



Fig. 3: Testis from male rat receiving 10^2 CFU *Bacillus thuringiensis*
Note: no visible lesion seen (H & E x 100)

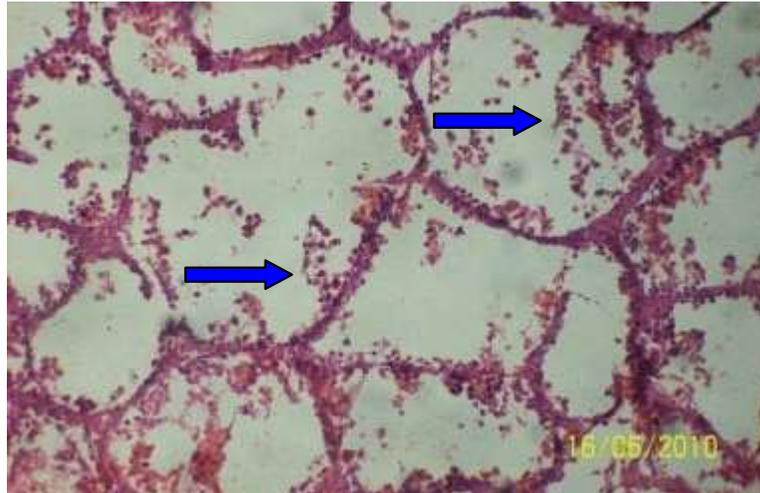


Fig. 4: A photomicrograph of a section of *B. thurigiensis* treated male rat's testis at 10^4 CFU showing severe germinal erosions (H & E x 100)

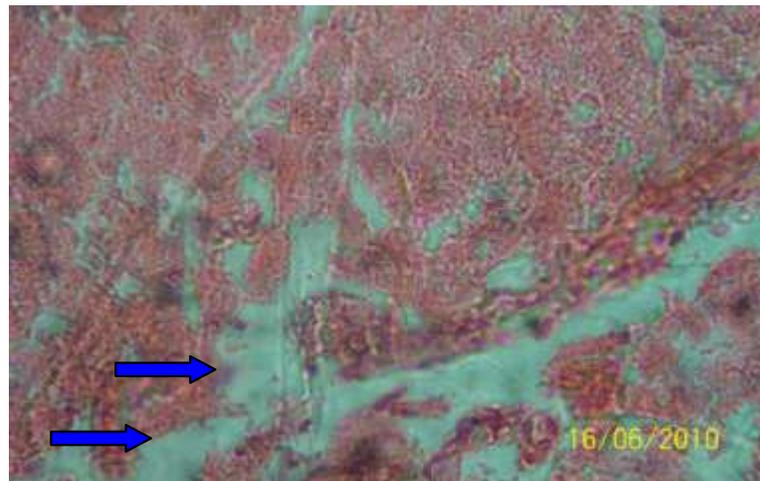


Fig. 5: A photomicrograph of a section of bacteriocin *B. thurigiensis* treated male rat's liver at 10^4 CFU showing periportal hepatic necrosis (H & E x 100)

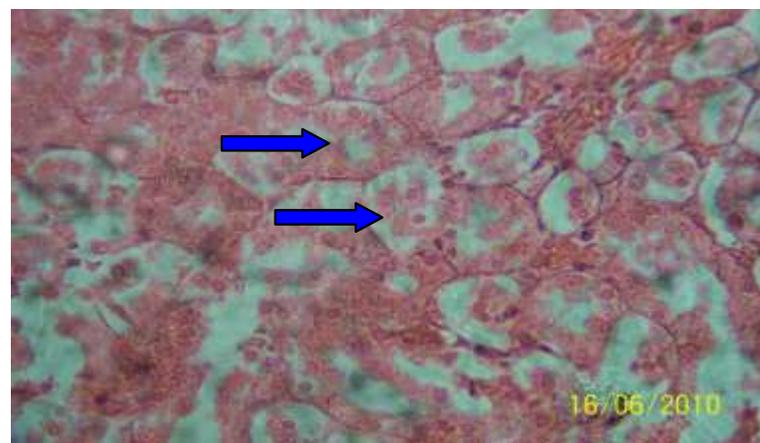


Fig. 6: A photomicrograph of a section bacteriocin of *B. thurigiensis* treated male rat's kidney at 10^4 CFU showing severe diffuse tubular necrosis (H & E x 100)

Histopathological Findings: Male and female rats given *B. thuringiensis* at 10^2 CFU, female rat at 10^4 CFU and female rat given the bacteriocin of the organism at 10^2 CFU and 10^4 CFU produced no adverse lesions in the internal organs. Male rat given *B. thuringiensis* at 10^4 CFU showed severe germinal erosions of the testis (Fig 4) and its bacteriocin at 10^4 CFU showed periportal hepatic necrosis of the liver (Fig 5) and diffuse tubular necrosis of the kidney (Fig 6).

DISCUSSION

The motilities observed could be due to higher doses that were used in the rats since such observation was not found at lower doses.

Increase in PCV, RBC and Hb is an indicator that the rats were not anemic while decrease level is a sign of anemia. PCV measures the percentage by volume of packed RBC in a whole blood sample after centrifugation [19] measures the amount of Hb in grams in 1dl of whole blood and provides an estimate of oxygen carrying capacity of the RBCs. From this studies, there is a reduction of PCV, erythrocyte and consequently Hb concentration at day 2 which may be attributed to more than one factor, that is the failure to supply the blood circulation with cells from haemohepatic tissues, since the liver has an important role in the regeneration of erythrocyte and the possible destructive effect on erythrocyte by the toxicant. The obtained results are in agreement with those found by Ali and Anubama *et al.* [20, 21] who stated that avermectins reduced erythrocyte, leucocyte counts and hemoglobin concentration in rabbits and rats. It may also be due to reduction of blood during sampling or hemolysis of blood cells due to the intramuscular administration of the *bacilli* strain. A significant increase in PCV, RBC and Hb by the bone marrow at day 10 could be due to regeneration of blood in the circulatory system since there were no sampling carried out at that interval and a reduction of stress due to reduced activities of the organism. This is useful in increasing the oxygen carrying capacity, hence increasing tissue oxygenation. WBC count is the number of WBC in a cubic millimeter of whole blood and is usually important in fighting against infections [16]. The significant decrease in WBC at day 2 from the hematological report indicates low level of infection in the experimental rats, or may be related to suppression of the production of the WBC resulting from reactions to substances [22]. A significant increase in the WBC at day 10 could be due to the presence of the *bacilli* organism which is foreign to the circulatory system so that they could destroy and

inhibit the activities of the intramuscularly injected organism and its bacteriocin in the system. From the result, the increase in neutrophil at day 2 which are usually the first cell to arrive at the site of infected cells showed that continuous use of these micro-organisms as biopreservatives to animals or humans, the principal function of phagocytes, which is to defend against invading micro-organisms by ingesting and destroying them, thus contributing to cellular inflammatory process may not be compromised. However, it can be deduced that as the experiment progresses the incidence of neutrophils in the blood circulation decreases. The significant increase in lymphocytes at day 10 may be due to their clearing activities of the cellular debris after the *bacilli* organism have been engulfed and destroyed by neutrophils. Increase in lymphocytes from this experimental result may go a long way to suggesting that *B. thuringiensis* and its bacteriocin must have influenced the defence mechanism of the test rats. So the continuous exposure of the body system of animals to this *Bacilli* organism may cause lymphocytosis, which may then account for its use as biopreservatives. Mean Corpuscular Volume (MCV) measures the average volume of a red cell in an individual's blood. From the haematological result, the significant increase in MCV at day 2 shows that the anemia is regenerative since there was a decrease in PCV and RBC at day 2 while at day 10, there were significant increase. Therefore, MCV is normocytic. MCH (Mean Corpuscular Hemoglobin) which measures the weight of hemoglobin in a red cell of an individual sample increases significantly as the experiment progresses. This shows that the anemia is normochromic and tissue oxygenation is increased.

The liver of the control rats showed normal structure of hepatic lobules and hepatocytes, hepatocytes form columns of cells adherent to each other by one or more surfaces. Bile canaculi were present in between two columns of hepatocytes. At least, one surface of any hepatocyte was in contact with liver sinusoids. The cytoplasm often appeared coarsely granular with empty vacuolated areas where lipid droplets have been dissolved during preparation of the section. The nuclei were spherical, centrally located and variable in size and with one nucleolus. Hepatocytes may be binucleated, the sinusoids were variable in diameter and lined by discontinuous sheet of endothelial cells with flat nuclei, kupffer cells also located in the sinusoidal walls (Fig 1) this picture is the same as described in Ross and Pawlina [23]. The histological structure of the renal cortex of the control group showed normal structure of both renal corpuscles and tubules. The renal corpuscles appeared as

dense rounded structures known as glomerulus's, surrounded by narrow spaces called Bowman. Bowman capsule consisted of an inner or visceral layer covering the glomerulus's and an outer layer or parietal layer become continuous with the wall of the proximal convoluted tubules (Fig 2). This picture is the same as described in Gartner and Hiatt [24].

Information on the effect of *B. thuringiensis* and its bacteriocin on the reproductive system are scarce.

The severe germinal erosions of the testis from this studies may be related to dose range The obtained result is in agreement with Aniaga *et al.* [25] in which testicular toxicity was reported in rats although this report was due to the administration of metabolic extracts of *Ficus thoningii* leaves. Periportal hepatic necrosis of the liver and diffuse tubular necrosis of the kidney from this experiment may be attributed to high concentration of the treatment. Many reports had elucidated that hepatocellular damage could be correlated with the disturbed enzymes activities. In this respect, Rodwell [26] observed that liver tissues which were famous for their rich contents of aminotransferases suffer markedly from their loss under many pathological condition. Tubular necrosis is also an indication of tubular disease as a result of leakage of protein into the lumen. In sum there were no toxic changes related to the administration of *Bacillus thuringiensis* in the female rats at 10^2 CFU and 10^4 CFU, its bacteriocins at 10^2 CFU and 10^4 CFU and in the male rat when *Bacillus thuringiensis* was given at 10^2 CFU.

CONCLUSION

Therefore, it can be deduced that female consumers are at less risk when compared to male consumers of products in which bacteriocins or organism of *Bacillus thuringiensis* is being incorporated. *Bacillus thuringiensis* appears to be possibly used as biopreservative at lower concentration.

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