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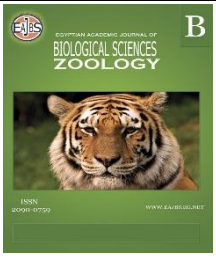

Mammalian Biosafety of *Bacillus thuringiensis* subsp. *kurstaki* -Based Bioinsecticide

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ABSTRACT

Bacillus thuringiensis subsp. *kurstaki* (*Btk*) is well-known and widely used as a biological insecticide. Registration of biopesticides with the national pesticides broad must be laboratory testing for acute toxicity as short-term and long-term health effects. Acute oral toxicity tests were carried out on rats with δ-endotoxin and formulation (9.4% WP) of *Btk* based on the Organization for Economic Co-operation and Development (OECD). The purpose of this study is to assess the biosafety of the bioinsecticide *Btk* (δ-endotoxin and 9.4% WP) in adult rats after a single oral dose based on hematotoxicity, hepato- renal toxicity, and the lipid profile. Results illustrate that most haematological parameters increased in treated rats. Compared to the control, liver enzymes (AST, ALT, and ALP) and globulin (Glb) concentration were increased and decreased in male rats subjected to both kinds of *Btk*. While female rats treated with both *Btk* products are showing a significant elevation in ALP, total protein (TP), albumin (Alb), and Glb levels versus the levels of ALT and AST. Urea concentrations decreased significantly in all treated rats, but Creatinine levels appeared to remain unchanged. Moreover, there was a decline and elevation in the serum of lipid profiles (T cholesterol and HDL) in treated male rats and female rats, respectively. LDL levels increased in treated male animals, while they decreased in treated females. All treated animals displayed negligible modifications in tissue somatic index, except a slight increase in lung weights when compared to the control. Contradictory, the body weight gain levels were reduced in all treated animals compared to untreated. In addition, there are no mortality or pathogenicity symptoms in all treated animals. These findings suggest that both treatments can induce hepato- renal toxicity and hematotoxicity with side effects on lipid profile aspects. Therefore, additional research, including subacute and chronic studies, is recommended.

INTRODUCTION

It's worth noting that pathogens including bacteria, fungi, viruses, nematodes, and

protists are one of the most common alternative pest control strategies (Ahirwar *et al.*, 2013). Microbial pesticides or biopesticides have an essential role in pest management due to their combat efficacy against a wide range of pests, safe to non-targeted organisms, and eco-friendly agent versus traditional pesticides (Saruhan, *et al.*, 2015**)**. *Bacillus thuringiensis* (*Bt*), a bacterial insect pathogen, has been thoroughly investigated for its potential to be an efficient biocontrol agent against a wide range of pests (Palma, 2017). Last five decades, microbial *Bt*-based products have been commercially produced to control insect pests (Baum *et al.,* 1999). The order Lepidoptera, Coleoptera, and Diptera are the primary targets of Cry proteins; additionally, there have been reports of their effect against nematodes and hymenopterans (Domnguez-Arrizabalaga *et al.*, 2020). *Bt* biopesticide is as effective as chemical pesticides and fits within the pest management approach, which supports non-chemical pesticides (Matyjaszczyk 2018). *Bt* can produce insecticidal toxin proteins such as crystalline, proteinaceous (Yan, *et al*., 2007), and antifungal toxin Yvgo protein (Manns, *et al*., 2012). Most strains of Bt can produce cry toxins, which are responsible for the toxic effect (Rubio & Moreno, 2016). The oral intake of this biopesticide is considered one of the main routes of mammalian exposure owing to the excessive application or during the industry of this product. Although several studies have reported that Bt-based biopesticides are safe, other studies have demonstrated the potential toxicity concerns of Bt cry proteins in mammals (Rubio & Moreno, 2016). In the field of pest control for various pests at various plants, currently there are various biopesticides that are registered in Egypt such as *Bacillus megaterium*, *Bacillus subtilis Bacillus thuringiensis* subsp. *Kurstaki*, *Beauveria bassiana*, (Egyptian isolates) and more (APC, 2022). However, there has been very little research on the biosafety of these biopesticide formulations on mammals and ecosystems (Ali et al., 2022; Farag, et al., 2022), except for a few papers that showed the side effects of isolated Bacillus thuringiensis on mammals, such as El-Saadany, et al (2006 and 2012). Therefore, in the current study, the oral toxicity of a biopesticide *Btk* (δ-endotoxin and formulation (9.4% WP) in rats was evaluated based on the hematotoxicity, and biochemical analysis. The findings of the current research will be a fundamental tool in determining the risk of exposure to Btk biopesticide.

MATERIALS AND METHODS

Bacillus thuringiensis Strain:

In this investigation, *Bacillus thuringiensis* subsp. *kurstaki* formulation (9.4% WP) was provided by Bioinsecticides Production Unit (BPU), Plant Protection Research Institute (PPRI), ARC, Ministry of Agriculture, Egypt.

Cultural Conditions of *Btk*:

Attathom *et al.,* (1995) described the *Btk* culture conditions that were carried out on T3 medium. The T3 medium containing tryptose 2.0g, yeast extract 1.5g, tryptone 3.0g, NaH_2PO_4. H_2O 8.9g and $MnCl_2$ 0.005g were prepared. The pH was adjusted to 6.8 and distilled water was added to complete the volume to 1 litre. After sterilization at 121^oC for 20 min, the medium was inoculated with inoculum. The inoculated flask was incubated on a shaker, 142 rpm at 30 ^oC for 72 hr. The number of spores/ml of the suspension was counted by plate count method, and then the broth was stored at 4˚C until use.

Preparation of *Btk* Cry Toxin:

The Cry toxin was prepared by using the procedure described by Mousa and Gujar (2005). Cells were growing in nutrient broth containing 50 µg/ml ampicillin for 72 h. Cells were harvested by centrifugation at 10000 rpm for 20 min at 4 ^oC and the pellet was washed three times by Cry wash solution (1 M NaCl containing 0.1 % triton X100) by

centrifugation for 10 min using 10000 rpm at 4ºC. Finally, the pellet was solubilized in solubilizing buffer (50 mM sodium carbonate pH 9.6, containing 0.1 % mM 2-mercaptoethanole and incubated at 37 ºC for 2 h with 150 rpm shaker, followed by centrifugation at 15000 rpm for 25 min to isolate the cell debris. The concentration of the toxin was measured by total protein kit, adjusted, and stored at -20ºC till further use (for more details, Asker and El Saadany, 2013).

Animals:

Eight to ten weeks old Sprague Dawley (SD), *Rattus norvegicus albinus* rats were obtained from the Egyptian Company for Biological Products and Vaccines (Helwan Farm). Thirty adult rats from each sex (male and female) were kept at laboratory conditions of 12 h light / 12 h dark and 22 ± 3 °C with 70 ± 5% relative humidity (RH). Prior to the trial, the animals were supplied with a commercial diet and water *ad libitum* for two weeks to let them adapt. One group of each sex was used to evaluate the oral LD_{50} of both *Btk* types (δ-endotoxin and 9.4% WP). The other group (males and females separately) was used for the biosafety studies by acute oral toxicity of both *Btk* types observing the physiological status of the animals.

Estimation of Oral LD_{50}:

The oral LD_{50} was estimated according to OECD (2008). A single sublethal dose with 0.5 ml/kg BW of δ-endotoxin *Btk* and 1 ml/kg BW of WP *Btk* (9.4% WP, containing 1.1×10^{10} spores) were orally administered to the animals used in the experiment, separately. Before selecting whether and how much to administer the following animal, each animal was carefully monitored for up to 48 hours. If the animal dies, a test will be carried out to determine the LD_{50}. If the first animal survives, two more were provided for the experiment. If both animals survive, the LD_{50} will exceed the maximum dose, then the experiment is over (carried out to full 21-day observation without dosing of further animals). Then the acute LD_{50} values were estimated.

Acute Oral Toxicity Study:

The biosafety study was performed according to USEPA (1996). The acute oral toxicity experiment was conducted as described previously (Ali *et al.*, 2022, Farag, *et al.*, 2022). Five animals of each sex for both *Btk* types (δ-endotoxin and 9.4% WP), separately were administered with a single dose of crude *Btk* (0.5 ml/100 g BW) and 1 ml WP/100 g BW (containing 1.1×10^{10} spores). Ten animals (5 rats from each sex) were used for control. The animals were weighed, killed, and dissected after the experiment periods terminated. From both treated and untreated animals, several essential organs (liver, kidney, brain, spleen, heart, lung, and testes) were gently removed, promptly washed with saline solution (0.9% NaCl), and next dried and weighed separately (absolute organ weight). As stated by Stanley *et al.*, (2005) and Nelli *et al.*, (2013), the tissue somatic index (the relative organ weight) was determined. The percentage of body weight changes was calculated by using the formula of Mansour *et al*., (2008).

Blood Sample Collection:

At the terminal of the experiment, the blood sample was collected from the retro-orbital plexus for hematological examination (Complete blood count, CBC) into an EDTA-treated tube (commercially available). Additionally, extra blood samples from each animal were obtained and placed in clean, dry, non-heparinized centrifuge tubes in accordance with (Schermer, 1967). The blood samples were centrifuged at 3600 rpm for 15 minutes after coagulation at room temperature for around 20 min to get the serum. The supernatants were placed into dry, clean tubes with caps, and they were kept at -40 °C until the biochemical analysis was conducted.

Haematological Determination:

The haematological analysis method was used as reported by Theml *et al.*, (2004).

The platelets (Plt), red blood corpuscles (RBCs), white blood corpuscles (WBCs), haemoglobin (Hgb), haematocrit (Hct), and red cell indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)], and red cell distribution width (RDW) are all measured as haematological profiles. Values for the erythrocyte indices were determined using the formula of (Schalm *et al.*, 1975).

Biochemical Assays:

The liver and kidney functions in serum were identified using commercial diagnostic kits. Based on (Reitman and Frankel, 1957; Roy, 1970), the aminotransferases [including alanine (ALT) and aspartate (AST)], and alkaline phosphatase (ALP) activity were assessed. In addition, total protein (TP), albumin levels (Alb) and globulin concentrations (Glb) were evaluated (Bradford, 1976; Doumas *et al.*, 1971). For renal function, creatinine and urea levels were assessed (Siest *et al.*, 1985; Fawcett and Scott, 1960).

Ethical Statement:

The ethical procedures and policies used in this experiment have been approved by the experiment protocols with (No. ZU-IACUC/2/F/156/2020) by the committee of Zagazig University's Institutional Animal Care.

Statistical Analysis:

The software AOT425 StatPgm was used to calculate the oral LD_{50} values for both crude and formulation *Btk*. SPSS (version 25, Chicago, USA) was used to analyse all data as means ± S.E. The differences between means were assessed by ANOVA one-way test and the Duncan multiple tests. The significant data represented in this study were considered at $P \leq 0.05$, 0.01, and 0.001 difference from the control. The data were represented as significant (*), high significant (**), and very high significant (***).

RESULTS

Acute Oral LD_{50} Study:

The acute LD_{50} values of *Btk* in rats were estimated. They were over 5000 mg/kg BW and higher than 1.1×10^{10} spores/kg with δ-endotoxin and formulation (9.4% WP) of *Btk*, respectively. Furthermore, none of the studied animals showed signs of infection, pathogenicity, mortality, or toxicological effects.

Acute Oral Toxicity Study:

Haematological Studies:

Haematological analyses were displayed in (Table 1a, b). In the δ-endotoxin - treated males, WBC, Plt, and RDW levels all dramatically reduced while RBCs, Hgb, and Hct levels slightly increased. In contrast, only RDW and Hct values significantly decreased in the formulation-treated males (Table 1a). In addition, the treated females revealed a significant increase in Plt, RBC and Hgb values **(Table 1b)**. Otherwise, no significant changes in WBC, Hct, MCH, MCHC and RDW levels activated in all treated females compared to controls.

Biochemical Studies:

The results of liver function biomarkers in Table 2a demonstrated that ALP, TP, Alb and Glb levels were increased in the male rats exposed to δ-endotoxin *Btk* compared to the untreated group. Additionally, ALT, TP, Alb, and Glb values were elevated in male rats exposed to WP- *Btk*. While the aminotransferase AST was significantly inhibited in treated rats. As shown in Table 2b, Female rats treated with both *Btk* products exhibited a significant elevation in TP, Alb, and Glb levels. Contrary, ALT values were dramatically reduced in treated females. As well as a slight decline was found in AST level in both types of *Btk* of treated females. ALP level was significantly elevated in females treated

with δ-endotoxin *Btk* when compared with the control group.

Table (1a): Effects of *Btk* (δ-endotoxin and Formulation) on haematological parameters of male rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
WBCs (X 10^3/µl)	6.2784 ± 0.2297^{b}	$5.000 \pm 0.0837^{ac***}$	6.262 ± 0.2270^{b}
Plt (10^3/mm^3)	790.0 ± 18.41^{b}	$494.4 \pm 10.88^{ac***}$	771.2 ± 12.19^{b}
RBCs (X 10^6/µl)	6.492 ± 0.0806^{b}	$6.778 \pm 0.0840^{ac*}$	6.358 ± 0.0383^{b}
Hgb (g/dl)	14.620 ± 0.1020^{b}	$15.568 \pm 0.3483^{ac*}$	14.268 ± 0.1592^{b}
Hct (%)	38.29 ± 0.1453^{bc}	$39.87 \pm 0.3103^{ac**}$	$37.08 \pm 0.5193^{ab*}$
MCV (fl/cell)	59.22 ± 1.084	60.67 ± 0.1592	57.20 ± 1.045
MCH (Pg/cell)	22.52 ± 0.1562	22.93 ± 0.2331	22.43 ± 0.1853
MCHC (g/dl)	38.12 ± 0.4609	37.88 ± 0.4128	39.32 ± 0.6591
RDW (%)	22.88 ± 0.1393^{bc}	$22.06 \pm 0.3750^{ac*}$	$21.29 \pm 0.1264^{ab***}$

n = 5, (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

Table (1b): Effects of *Btk* (δ-endotoxin and Formulation) on haematological parameters of female rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
WBCs (X 10^3/µl)	7.538 ± 0.2542	8.300 ± 0.2214	7.624 ± 0.3092
Platelet (10^3/mm^3)	665.6 ± 23.82^{b}	$779.6 \pm 4.297^{ac***}$	676.9 ± 15.94^{b}
RBCs (X 10^6/µl)	6.292 ± 0.0224^{bc}	$6.504 \pm 0.0507^{a**}$	$6.58 \pm 0.0490^{a***}$
Hgb (g/dl)	14.20 ± 0.2191^{c}	14.480 ± 0.1744^{c}	$15.280 \pm 0.1020^{ab***}$
Hct (%)	39.10 ± 0.5013	38.81 ± 0.2135	40.12 ± 0.3368
MCV (fl/cell)	63.18 ± 1.139^{b}	$60.30 \pm 0.0949^{ac*}$	63.28 ± 0.5142^{b}
MCH (Pg/cell)	22.58 ± 0.2975	22.20 ± 0.0949	23.28 ± 0.3072
MCHC (g/dl)	35.72 ± 0.2035	36.80 ± 0.0949	36.82 ± 0.7255
RDW (%)	21.90 ± 0.2225	22.53 ± 0.1903	21.89 ± 0.1629

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

Data collected from renal functions analysis (Fig. 1) revealed that the urea concentration was dramatically reduced in treated male and female rats with both *Btk* types compared to the control. On the other hand, the creatinine levels were not significantly altered in both treated male and female rats (Fig. 1).

Table (2a): Effects of *Btk* (δ-endotoxin and Formulation) on liver function of male rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
ALT (U/l)	21.68 ± 0.9293^{c}	23.34 ± 0.9410^{c}	$27.91 \pm 1.0587^{ab***}$
AST (U/l)	28.62 ± 1.273^{bc}	$13.53 \pm 0.6113^{a***}$	$12.11 \pm 0.5480^{a***}$
ALP (U/l)	122.0 ± 1.624^{bc}	$190.4 \pm 3.001^{ac***}$	$82.71 \pm 2.567^{ab***}$
TP (g/dl)	12.98 ± 0.2919^{bc}	$20.19 \pm 0.6721^{ac***}$	$24.27 \pm 0.8931^{ab***}$
Alb (g/dl)	7.399 ± 0.0442^{bc}	$11.89 \pm 0.3533^{ac***}$	$17.23 \pm 0.6201^{ab***}$
Glb (g/dl)	5.582 ± 0.2819^{bc}	$8.306 \pm 0.3637^{ac***}$	$7.043 \pm 0.2956^{ab***}$

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

Table (2b): Effects of *Btk* (δ-endotoxin and Formulation) on liver function of female rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
ALT (U/l)	29.94 ± 0.952^{bc}	$18.42 \pm 0.813^{a***}$	$20.57 \pm 0.9813^{a***}$
AST (U/l)	44.94 ± 1.9910^{c}	40.88 ± 0.8530	$39.86 \pm 1.154^{a*}$
ALP (U/l)	112.3 ± 3.624^{b}	$162.4 \pm 7.029^{ac**}$	109.3 ± 2.868^{b}
TP (g/dl)	12.48 ± 0.5237^{bc}	$18.54 \pm 0.6317^{a***}$	$19.33 \pm 0.6239^{a***}$
Alb (g/dl)	8.857 ± 0.3342^{bc}	$11.80 \pm 0.3994^{a***}$	$11.95 \pm 0.3730^{a***}$
Glb (g/dl)	3.627 ± 0.1994^{bc}	$6.745 \pm 0.2590^{a***}$	$7.381 \pm 0.3233^{a***}$

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

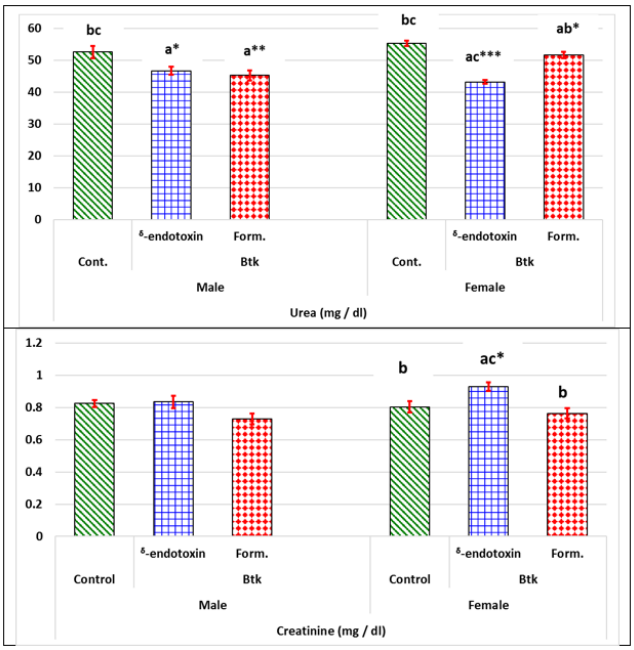

Fig. (1): Effects of *Btk* (δ-endotoxin and Formulation) on kidney function of male and female rats after acute exposure.

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001. a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05.

Data displayed in Tables 3a and 3b elucidated the analysis of lipid profile in male and female rats treated with both products of *Btk*. It is worth noting that triglyceride and VLDL (Table3a) values didn't change in any of the treated rats compared to the control group. In δ-endotoxin *Btk*-treated males, Serum cholesterol and HDL were reduced compared to the control. In contrast, LDL levels were markedly raised in both types of *Btk*-treated males compared to untreated males. On contrary, δ-endotoxin *Btk*-treated females exhibited higher cholesterol and HDL values than untreated females, but LDL levels diminished (Table 3b). Interestingly, females exposed to WP- *Btk* didn't exhibit any significant alterations in the values of lipid profile parameters.

Table (3a): Effects of *Btk* (δ-endotoxin and Formulation) on lipid profile of male rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
T. Cholesterol (mg/dl)	117.2 ± 0.7275^{b}	$109.2 \pm 0.5957^{ac***}$	116.8 ± 0.7595^{b}
Triglyceride (mg/dl)	71.42 ± 1.572	72.67 ± 2.458	69.01 ± 0.2301
HDL (mg/dl)	94.15 ± 0.7008^{bc}	$73.37 \pm 0.6839^{ac***}$	$63.13 \pm 0.6217^{ab***}$
LDL (mg/dl)	8.745 ± 0.4469^{bc}	$21.34 \pm 1.049^{a***}$	$39.89 \pm 1.058^{a***}$
VLDL (mg/dl)	14.28 ± 0.3144	14.53 ± 0.4916	13.80 ± 0.0460

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

Table (3b): Effects of *Btk* (δ-endotoxin and Formulation) on lipid profile of female rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
T. Cholesterol (mg/dl)	103.2 ± 0.9979^{b}	$110.3 \pm 1.205^{ac***}$	105.1 ± 0.6945^{b}
Triglyceride (mg/dl)	163.4 ± 4.989	154.2 ± 2.829	169.5 ± 4.369
HDL (mg/dl)	62.25 ± 0.2550^{b}	$76.84 \pm 0.9960^{ac***}$	62.11 ± 0.3840^{b}
LDL (mg/dl)	8.235 ± 0.418^{b}	$2.567 \pm 0.1370^{ac***}$	9.091 ± 0.3445^{b}
VLDL (mg/dl)	32.68 ± 0.9979	30.85 ± 0.5659	33.90 ± 0.8738

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

Body weight gain and relative organ weight studies:

Fig. 2 and Tables 4 illustrated the changes in body weight gain and tissue somatic index (relative organ weights) as the physiological status of the normal and treated animals. According to the findings in Fig. 2, treated animals with both *Btk* types had remarkably less progressive % BWG/week at the experiment termination compared to the weight at the beginning. However, the animals in the control group showed consistent progressive growth in BWG over the experiment period. Interestingly, Table 4a and 4b revealed that these products as bioinsecticides did not stimulate any significant alterations in relative organ weight of animals exposed to both types of *Btk* compared with untreated rats even though the treated animals exhibited a slight increase in body weight. Meanwhile, a modest raise was monitored in the tissue somatic index of lungs in treated male and female rats.

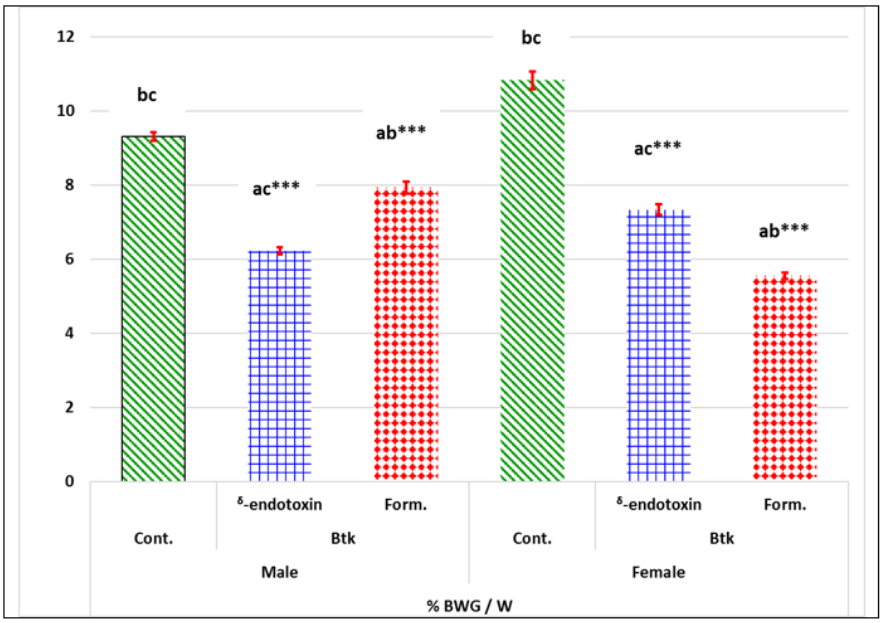

Fig. (2): Effects of *Btk* (δ-endotoxin and Formulation) on body weight gain of male rats after acute exposure.

% of weekly body weight gain = [(Final Bw. - Initial Bw) / (Initial Bw X No. of weeks)] x 100.
n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001. a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

Table (4a): Effects of *Btk* (δ-endotoxin and Formulation) on tissue somatic index (g/100gm bw) of male rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
Liver	9.058 ± 0.3695	8.953 ± 0.0647	9.158 ± 0.0835
Kidney	4.459 ± 0.1839	4.228 ± 0.0156	4.508 ± 0.0453
Brain	4.248 ± 0.0345	4.071 ± 0.0526	4.095 ± 0.0923
Spleen	2.894 ± 0.0895	2.869 ± 0.0781	3.038 ± 0.0490
Heart	3.210 ± 0.0537	3.1107 ± 0.0684	3.0243 ± 0.0354
Lung	3.859 ± 0.0358 ^{bc}	4.2839 ± 0.0976^{a***}	4.078 ± 0.0600 ^{a*}
Testes	5.584 ± 0.2487	5.793 ± 0.1223	5.900 ± 0.0663

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

Table (4b): Effects of *Btk* (δ-endotoxin and Formulation) on tissue somatic index (g/100gm bw) of female rats after acute exposure.

Parameters	Cont.	*Btk*	
		δ-endotoxin	Form.
Liver	9.320 ± 0.3809	10.11 ± 0.0073	9.492 ± 0.2537
Kidney	4.363 ± 0.0930^{b}	4.707 ± 0.0421^{a*}	4.470 ± 0.1076
Brain	4.940 ± 0.0347	4.908 ± 0.0119	4.815 ± 0.0796
Spleen	2.704 ± 0.0957^{bc}	3.099 ± 0.0528 ^{a*}	3.163 ±0.1334 ^{a**}
Heart	3.317 ± 0.1034	3.705 ± 0.1122	3.624 ± 0.1357
Lung	4.183 ± 0.2915^{b}	5.466 ± 0.3991^{a*}	4.879 ±0.1618

n = 5 (M ± SE), Form.: Formulation
*, ** and *** significant at P ≤ 0.05, 0.01 and 0.001.
a, b, and c, Significant difference versus Control, δ-endotoxin, and Formulation respectively, at p ≤ 0.05
The percentages were transformed into angular transformation values (arcsin √. percent).

DISCUSSION

The use of biological pesticides is a safer alternative to chemical pesticides for pest management (El-Bendary, 2006). Unfortunately, biopesticides may stimulate adverse impacts on mammals, and the environment (Prasad and Shethna 1975). This investigation assessed the possible toxicity of the microbial pesticide *B. thuringiensis* in rats. Our results of the oral toxicity investigation unveiled that no death, or toxicological features, were observed in any group during the experiment carried out with the tested *B. thuringiensis* subsp. *kurstaki* biopesticides.

Most strains of *B. thuringiensis* include cry toxins, which are toxic substances that affect a target organism (Rubio & Moreno, 2016). Conversely, our findings of oral toxicity studies didn't reveal any pathogenetic symptoms, the haematological analyses showed that these toxins could cause hematotoxicity in rats exposed to *Bt* biopesticides. Similarly, Eissa and Zidan (2010) reported that white blood cell counts are much lower than normal in male rats treated with abamectin and *Bt*. This response may be a symptom of immunosuppression (Schroder, *et al*., 2007). The elevation of haemoglobin concentration in treated animals may be attributed to the lung shrinking and consequently lower oxygen (Akunov *et al.*, 2018). All treated rats had altered platelet counts, which could be due to various triggers, such as tumours, inflammatory diseases, systemic infections, and haemorrhage (Tefferi and Barbui, 2017). The obtained results are consistent with those reported by Larki (2019) who stated that haematological abnormalities were triggered by the oral administration of a high dose of *Bt* biopesticide.

It is well known that the liver is the primary target where harmful substances that enter the body are detoxified. Consequently, liver function can serve as a proxy for xenobiotic toxicity (Balistreri and Shaw, 1987). In the current study, the hepatic biomarkers activity was dramatically increased after oral administration of *Bt* biopesticide in animals. AST and ALT are regarded as sensitive markers of hepatocellular damage and can restrictively offer a quantitative evaluation of the severity of liver damage (Peng, *et al.*, 2007). The biomarker ALP is the enzyme that can evaluate the validity of the plasma membrane (Zhou, *et al.*, 2017). Our findings indicated that liver damage and heart infections frequently result in a significant increase in serum ALP levels in treated rats with δ-endotoxin *Btk* (Wannhoff, *et al.*, 2017). The results of this investigation demonstrated that high dosages of this δ-endotoxin *Btk* biopesticide could cause hepatotoxicity in experimental rats by significantly altering ALT, AST, and ALP activity. The elevation of TP levels is likely due to the disorder in liver and kidney functions

(Mansour and Mossa, 2005). The present study showed that both *Btk* types had modest effects on liver function expressed as ALT and AST activity and TP. These results might support the theory that *Btk* is relatively safe which has been tested on liver functions, one of the most important organs in mammals.

The primary organ that is destroyed after exposure is the kidney owing to its multifunctional role in the removal of waste substances and poisons from blood (Mossa *et al*., 2018). The byproduct of protein metabolism; creatinine and urea, will have higher levels in renal failure (Stark, 1980). Regarding the reduction of urea concentration, it may be due to renal tubular dysfunction and nephrotoxicity (Walmsley and White, 1994), although the creatinine levels were not considerably altered.

A crucial element of lipids, cholesterol, functions in cell physiology as a catalyst for steroid hormones (Kuzu *et al.*, 2016). Free radicals mainly promote disease and death which result from cholesterol. Free radicals can be eliminated by natural antioxidants including plants, bacteria, fungi, and microbial metabolites (Zohri *et al*., 2017). The considerable reduction of cholesterol may be attributed to the hyperactivation of the thyroid (Rizos *et al*., 2011). The organ and body weight are used in toxicological research to gauge an organism's normal growth and development, metabolic condition, and side effects of various chemicals (Mossa *et al*., 2018). In the current research, the body weight gain of all *Btk*-treated animals was significantly reduced compared to the control group on the other hand the biopesticide had no remarkable effect on somatic tissues. A similar observation has been made after the treatment with the chemical pesticide chlorpyrifos (Ambali *et al*., 2007). The fact that lower weight gain could be attributed to altered metabolism, lower appetite, higher energy expenditure, or malabsorption (Talbot *et al*., 2020).

The results of the present investigation can be concluded that δ-endotoxin *Btk* causes more severe modifications than *Btk* formulation (9.4% WP). Moreover, the male rats were more negatively impacted than the females.

CONCLUSION

The current study verified the possible harmful effects of δ-endotoxin and formulation (9.4% WP) *Bacillus thuringiensis* subsp. *kurstaki* biopesticide. The high doses of the biopesticide *Btk*, particularly δ-endotoxin *Btk*, administered orally can cause various hepato- renal toxicity, and haematological problems in vertebrates like rats (males and females) with side effects on lipid profile aspects. Therefore, additional research, including subacute and chronic studies, is recommended. Moreover, Further investigations may be needed to evaluate some natural compounds that can attenuate biopesticide toxicity.

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ARABIC SUMMARY

السلامة الحيوية للثدييات من بكتريا الباثليس ثيورنجينيسيس كورسيتاكى *Bacillus thuringiensis* subsp. *kurstaki* - كمبيد حشري حيوي.

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بكتريا *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) معروفة جيدًا وتستخدم على نطاق واسع كمبيد حشري بيولوجي. ولتسجيل تلك مبيدات الآفات الحيوية لتستخدم على نطاق واسع فلابد أن تجرى اختبارات السمية المعملية لدراسة التأثيرات على الصحة العامة للإنسان سواء على المدى القصير أو على المدى الطويل. لذا في هذه الدراسة تم إجراء اختبارات السمية الفموية الحادة على الفئران بمستحضر *Btk* (9.4% WP) ودلتا اندو توكسين *Btk* δ-endotoxin وفقًا لمنظمة التعاون الاقتصادي والتنمية (OECD). والغرض من هذه الدراسة هو تقييم السلامة الحيوية للمبيد الحيوي *Btk* (*Btk* δ-endotoxin وWP) في الجرذان البالغة بعد جرعة فموية واحدة من خلال دراسة التأثير على صورة الدم الكاملة (CBC) والسمية الكبدية والكلوية والتأثير على صورة الدهون. توضح النتائج أن معظم متغيرات الدم زادت في الفئران المعالجة. وبالمقارنة مع المقارنة، إذ أن إنزيمات الكبد (AST ، ALT، ALP) والجلوبيولين (Glb) زادت وانخفضت في ذكور الجرذان المعرضة لكلا النوعين من *Btk*. بينما تُظهر إناث الفئران التي تم تجريعها بمنتجات *Btk* ارتفاعًا ملحوظًا في مستويات ALP والبروتين الكلي (TP) والألبومين (Alb) و Glb مقابل مستويات ALT و AST. كما انخفضت تركيزات اليوريا بشكل ملحوظ في جميع الفئران المعاملة، ولم يحث أي تغيرات في مستويات الكرياتينين. علاوة على ذلك، كان هناك انخفاض وارتفاع في الكوليسترول الكلى والدهون عالية الكثافة (Total Cholesterol و HDL) في سيرم ذكور الفئران المعالجة وإناث الجرذان على التوالي. كما زادت مستويات الدهون منخفضة الكثافة (LDL) في ذكور الحيوانات المعاملة، بينما انخفضت في الإناث المعالجة. وأظهرت جميع الحيوانات المعاملة تغيرات غير معنوية في المؤشر الجسدي للأنسجة، باستثناء زيادة طفيفة في وزن الرئة بالمقارنة مع مجموعة الكنترول. على النقيض من ذلك، انخفضت مستويات التغير في وزن الجسم في جميع الحيوانات المعالجة مقارنة بالحيوانات غير المعاملة. بالإضافة إلى ذلك، لا توجد أعراض مرضية أو نفوق في جميع الحيوانات المعاملة. تشير هذه النتائج إلى أن كلا صورتي البكتريا سواء *Btk* δ-endotoxin أو المستحضر (9.4% WP) يمكن أن يحفز التسمم الكبدي الكلوي والتسمم الدموي مع آثار جانبية على صورة الدهون. لذلك، نوصى بإجراء أبحاث إضافية، بما في ذلك الدراسات تحت الحادة والمزمنة.