The effect of Teflubenzuron and *Bacillus thuringiensis* on some haematolgical parameters of albino rats.

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Abstract: Astudy to evaluate the toxicological and haematological effects of the entomopathogen (*Bacillus thuringiensis kurstaki*) and the insect growth regulator (Teflubenzuron) on albino rats. Administration of *B. thuringiensis Kurstaki* for 12 weeks to rats at dosages of 10000 mg/ kg/ day did not produce toxic effects. The effects of *B. thuringiensis Kurstaki* body weight showed an insignificant changes in body weight, liver, kidney and testicular weights as compared to the levels of the control group. On the contrary, Teflubenzuron caused a significant decrease in body weight of rats, increased liver weight but the kidney weight was decreased. In addition there was a slightly decrease in testicular weight as compared to the level in the control group. It was concluded that *B. thuringiensis* hadnot any significant effect on the haematological parameters.
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Key words:- *B. thuringiensis Kurstaki*- Teflubenzuron- albino rats- haematolgical parameters.

Introduction:

Natural pesticides and biopesticides are the prospective solutions to avoid the deteriorating effects of synthetic pesticides. Recently, the wide used of biopesticides in agricultural and public health programs has advers health effects on human and animal. Therefore, it is necessary to focus on studying the detrimental effects of such natural pesticides and biopesticides on mammals. Mammalian safety studies were carried out with *Bacillus thuringiensis kurstaki* orally administered to rats. The clearance and distribution of *B. thuringiensis* were evaluated. The results confirmed the safety of *Bacillus thuringiensis kurstaki* to rats (Tsai et al, 1995). The hazardous effects of the benzoylphenylurea on mammalian tissues are still under investigation and works on it are centered mainly on controlling insect production. Administration of benzoyl-phenylurea resulted in altered enzyme activities of rat liver, renal damage, and reproductive disorders to experimental animals (Karim, 1998; Husien 2006).

The present study aimed to evaluate the toxicological and hematological effect of the entomopatogen (*Bacillus. thuringiensis* subsp. *kurstaki* and one insect growth regulator (Teflubenzuron) on some hematological parameters of albino rats.

Materials and Methods:

I-Source and rearing of albino rats:-

A total of forty male albino rats (*Rattus norvegicus*) weighing 120-150 gram each were used. The rats were obtained from the Farm of Central Organization of Serum & Vaccine (Abasia Farm, Egypt). Rats were allowed to be acclimatized to laboratory condition for two weeks prior to the experiment. The rats were housed in plastic cage under hygienic condition in dry-bulb temperature 18-20 ^{o}C and fed on a commercial pellet diet and barley (natural ingredient diet). The diet includes protein, minerals, vitamins, energy resources and other beneficial dietary constituent as recommended by **(National Research Council (NRC). 1995)** and the diet of the rats of the present study was supported with Soya Bean. The water supply was evaluated as recommended as 1/4-1/3 of their body weight in water daily. Food and water were available all over the experimental period.

2- Chemicals used:

1-Teflubenzuron (NO Molt) chitin synthesis inhibitors IUPAC1- (3,5-dichloro-2,4-difluorophenyl)-3-(2,6 difluorobenzoyl) urea.

2-*Bacillus thuringiensis* subsp. *kurstaki* (Protecto®) was obtained from Plant Protection Research Institute Biopesticide Unit Production.

III-Experimental design of albino rats:

The animals were arranged into three groups, each composed of 10 individuals as follows:

Control saline – treated rats group, *Bacillus thuringiensis* treated rats group, Teflubenzuron treated rats group. After the end of each week blood samples were taken. *Bacillus thuringiensis kurstaki* was suspended in saline solution and intragastrically administered by stomach tube with different large doses but it was observed that it had no effect on the studied rats. *Bacillus thuringiensis kurstaki* was supplied at the rate of 10000 mg/kg body weight from the commercial product to evaluate the sub chronic effect of Bt at dose a higher than that reported in previous studies. Teflubenzuron (benzoylphenylurea) was supplied at a concentration of 10%. The applied dose of Teflubenzuron was 105 mg/kg body weight (equivalent to 0.1 of LD_{50}). It was dissolved in saline solution, then administered intra gastrically by stomach tube every other day for 3 months The drug was freshly prepared prior to every treatment.

Blood samples were obtained from the retro-orbital plexus and the tail using 21-gauge needle of overnight fasted rats. Blood was collected into heparinized tube for assay of the complete blood picture**.**

Results And Disscussion:

1. Toxicological effect of Teflubenzuron on rats:-

Rats treated with Teflubenzuron at 1/10 of its $LC_{50,}$ developed clinical symptoms, which were progressing by time marked distension of the abdomen. This was the only clinical symptom observed in rats after the first two weeks of treatment. In the third week, rats lost their vitality and activity. Some rats developed nervous manifestation and moved in circles. During the remaining weeks of the experiments, general weakness and cachexia were observed. The animals were reluctant to move and showed nervous manifestation and hurried respiration. Also the results of **US EPA (1998)** were in agreement with the obtained observations concerning a study on chronic rat feeding with flufenoxuron which identified the following effects: seizures, including seizures resulting in death.

2. Toxicological effects of Bacillus thuringiensis on rats:-

Administration of *B. thuringiensis Kurstaki* for 12 weeks to rats at dosages of 10000 mg/ kg/ day did not produce toxic effects. The effects showed an insignificant changes in body weight, liver, kidney weight and testicular weights of rats as compared to their levels in their control group as recorded in table (1). Results are in agreement with those reported by PIP (2006). Where the $LD_{50}$was is greater than 5000 mg/kg for the *B. thuringiensis Kurstaki* product Javelin in rats and greater than 13,000 mg/kg in rats exposed to the product. Single oral dosages of up to 10,000 mg/kg that did not produce toxicity in mice, rats, or dogs. The dermal LD_{50} for a formulated *B. thuringiensis Kurstaki* product in rabbits was adminstrated by 6280 mg/kg. A single dermal application of 7200 mg/kg of *B. thuringiensis* was not toxic to rabbits. Ray, (1991) reported that dietary administration of *B. thuringiensis* for 13 weeks to rats at dosages of 8400 mg/kg /day, did not produce toxic effects. Some reversible abnormal redness of the skin was observed when 1 mg/kg/day of formulated *B. thuringiensis* product was put on scratched skin for 21 days.

Also, the above results are in agreement with the results of, Tsai, et al. (1995) who reported that mammalian safety studies were carried out with *B. thuringiensis* (*kurstaki)* orally administered to rats. Neither clinical symptoms nor histopathological changes were detected during the test. The total number of colony-forming units (CFU) recovered was less than in the initial inoculation. No spore germination was observed in the tissues after administration. The results confirmed the safety of *B. thuringiensis kurstaki* to rats. Also the results of Roe, (1991) British Columbia Ministry of Health. (1992), Washington State Department of Health (1993), Salamitou *et al*. (2000), seem on line with the present results.

2.1 Hematological effects on rat:

Data presented in table (2) indicated that prolonged administration of IGRs (three weeks) affected hematological parameters in rats while *Bacillus thuringiensi*s recorded insignificant results on the hematological parameters. Teflbenzuron affected these parameters by decreasing Hb% (14.31 g/dl in the 1st week to 7.29 in the 3rd week), RBC, s count ($5.11x10^6$celllmm in the 1st week to 2.8 $x10^6$celllmm in the 3rd), Hematocrite% (Hct%) (43% in the 1st week to 21% in the 3rd week), mean corpuscular volume (MCV) (87*Fl* in the 1st week to 78*Fl* in the 3rd week), mean corpuscular haemoglobin concentration (MCH) (28.03*pg* in the 1st week to26.3*pg* in the 3rd week) and platelets (430.36 $x10^3$cell/min in the 1st week to 280.66 $x10^3$cell/min in the 3rd week) except the leucocytes (WBCs) that showed significant increase, the result of the defense role of WBC, S against toxic action of Teflubenzuron. These results are in agreement with *El-sherbiny et.al (1995)* recording decrease in erythrocyte counts and packed cell volume.

Table 1. Effect of *B.thuringiensi*s and Teflubenzuron on body weight and some organs weight of rats.

Weight in gm. Mean ±SD	**Control**	***B.thuringiensis***	**Teflubenzuron**
Body weight	255±11.78	249±11.8	226.4±3.22
Liver weight	9.9±0.47	9.7±0.45	11.47±0.52
Kidney weight	2.95±0.163	2.75±0.163	2.16±0.16
Testis weight	2.67±0.025	2.65±0.03	2.08±0.08

Table 2. Effect of *B.thuringiensi*s and Teflubenzuron on some hematological parameters of albino rats.

Hematological parameters		**control**	**4 weeks**		**8 weeks**		**12 weeks**	
			B.thuringiensis	**Teflubenzuron**	***B.thuringiensis***	**Teflubenzuron**	***B.thuringiensis***	**Teflubenzuron**
Hb g/dl	Mean	16.13	15.72	14.31	15.72	10.74	15.5	7.29
	SD	2.66	2.45	2.47	2.45	2.68	2.66	2.32
RBCs 10^6celllmm	Mean	5.91	5.32	5.11	5.88	4.4	5.23	2.8
	SD	1.23	.043	.014	.043	.07	1.23	1.24
Hct%	Mean	48	46	43	46	30	48	21
	SD	2.3	2.1	2.3	2.1	1.9	2.3	2.41
MCV *Fl*	Mean	89	87	87	87	79	89	78
	SD	5.32	5.44	4.79	5.44	5.32	5.32	4.98
MVH *pg*	Mean	29.7	28.3	28.03	28.3	24	29.7	26.3
	SD	2.03	1.69	2.36	1.69	1.7	2.03	1.6
WBCs 10^3cell/mm	Mean	5.99	6.99	6.6	5.78	5.75	7.34	7.33
	SD	1.98	1.55	1.25	2.55	1.28	3.29	1.27
Platletes 10^3cell/mm	Mean	450.5	453.2	430.36	453.2	340.2	450.2	280.66
	SD	98.33	96.8	115	97.9	152.3	94.78	96.3

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