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HAEMATOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL ALTERATIONS INDUCED BY ABAMECTIN AND *BACILLUS THURINGIENSIS* IN MALE ALBINO RATS

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The renal- and hepato-toxicity induced by abamectin pesticide (Vertimec) and a commercial form of a bio-insecticide *Bacillus thuringiensis* (Agerin) in male albino rats were evaluated. Blood picture and blood glucose level were investigated. Male albino rats were administered dietary doses each equivalent to 1/10 or 1/100 of the LD_{50} values of each toxicant for 30 consecutive days. Abamectin was found to pose risks of renal- and hepato-toxicity in rats, since the biochemical parameters of liver function (i.e. aspartate aminotransferase activity, alanine aminotransferase activity, acid phosphatase activity, albumin, and total protein levels) and kidney function (uric acid and creatinine concentration) were severely affected. These effects were verified by histopathological examination of liver and kidney tissues. Likewise, some haematological indices (i.e. erythrocyte count, leukocyte count and haemoglobin concentration) were also influenced; in addition abamectin might cause hypoglycaemia. On the other hand, the above-mentioned lesions were less pronounced in the case of *Bacillus thuringiensis*-treated rats.

Keywords: Abamectin – *Bacillus thuringiensis* – rats – toxicology

INTRODUCTION

Increased public concern of the potential adverse environmental effects associated with the heavy use of chemical pesticides has prompted the examination of alternative methods for pest control. Two of the promising alternatives are the use of abamectin which is a natural product pesticide isolated from the fermentation of the soil actinomycete, *Streptomyces avermitlis* and the microbial insecticide *Bacillus thuringiensis* (Bt). Abamectin which has potent acaricidal, insecticidal and anthelmintic properties in animals [25, 38, 39, 44], acts by stimulating the pre-synaptic release of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), binding to the post-synaptic receptors (i.e. GABA agonist) and thus disturbing Cl^- passage through the end plate resulting in inhibitory post-synaptic potentials and eventual paralysis [8, 28]. *Bacillus thuringiensis* is a ubiquitous gram-positive soil bacterium, and has toxicity against a wide spectrum of lepidopteran insects [15, 36]. Bt produces a parasporal inclusion body during sporulation usually referred to as a crystal. This

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crystal is made of proteins and exert its mode of action by dissolving in the alkaline environment of the host insect midgut causing lysis of the midgut epithelial cell membranes, eventually leading to death of the larvae [16, 20, 23]. Therefore, the main target of the present study was to investigate their side effects on some haematological, hepatic and renal parameters as well as histopathological changes in liver and kidney of white albino rats.

MATERIALS AND METHODS

Pesticides used

Vertimec (Abamectin) 1.8% E.C.: a mixture containing a minimum of 80% avermectin B_1a (5-O-demethylavermectin A_1a) and a maximum of 20% avermectin B_1b (5-O-demethyl-25-de-(1-methylpropyl)-25-(1-methylethyl) avermectin A_1a) was obtained from MSD Sharp & Dohme GmbH, Germany.

Agerin *(Bacillus thuringiensis)* 6.5% W.P was purchased from Biogro International, Egypt.

Test animals

Adult male albino rats (Sprague-Dawely), *Rattus norvegicus* var. *albinus,* weighting 110–120 gm were purchased from the Biological Products & Vaccines Holding Company, Helwan Farm, Cairo, Egypt. Rats were kept under the laboratory conditions of 25 ± 5 °C and 65 ± 5% R.H., two weeks as an acclimatization period. They were housed in metal cages (35 × 25 × 20 cm) and maintained on *ad libitum* diet and water. This diet contained all the dietary needs and was obtained from Alexandria Oil and Soap Company, Ghamra, Cairo, Egypt. Animal experiments and housing procedures were performed in accordance to the animal care rules and they were approved by the authorities of the University.

Assessment of the oral LD_{50} for the tested pesticides

Preliminary investigation was carried out to calculate the median lethal dose (LD_{50}) for the tested pesticides to male albino rats. Four groups of rats, each of five individuals (n = 5) were used for each toxicant. Rats of each group were given orally the specified dose for each toxicant. Doses were prepared in corn oil. Oral dosing was done by a special syringe that has a needle equipped with a ball tip. Mortality counts of rats were recorded after 24 hours of treatment. The LD_{50} values were calculated according to the statistical method of [45]. Results demonstrated that, the oral LD_{50} values of Vertimec and Agerin to male albino rats are 18.1 and greater than 3000 mg a.i./kg body weight, consecutively.

Preparing of the toxicated diet

To calculate the quantities of pesticides required to be mixed with diet, preliminary experiments were performed to determine the quantity of the diet normally consumed by each rat per day. Ten male rats were caged individually, starved and each was offered adequate weighed quantity of diet. After 24 h, the residual quantity of diet was thoroughly collected and weighed. Average weight of diet consumed daily by each rat was calculated. Determination of individual feed consumption was determined periodically according to ages and body weights of the tested animals during the experimental period. The desired quantity of each pesticide was diluted in a proper volume of water and mixed thoroughly with diet. The mixture was allowed to dry at room temperature and then kept in a deep freezer till being used. This mixture was considered a fresh treated diet for three days; afterwards, new mixtures were periodically prepared by the same manner. The ratio of pesticide/diet in the mixture was calculated on the basis that toxicated diet ingested by an animal/24 h, should carry the required quantity of a pesticide that is nearly equivalent to the desired dose calculated as fraction of LD_{50} of this pesticide. Variations of body weights of animals through the experimental period were taken in consideration. Freshly prepared toxicated diet and clean water were offered daily to rats *ad libitum*, for 30 consecutive days.

Experimental design

Rats were divided randomly into five groups, each of five rats. The first and second groups received diet containing abamectin at quantities equivalent to 1/10 and 1/100 of the LD_{50} values. The third and fourth groups received diet containing *Bacillus thuringiensis* at quantities equivalent to 1/10 and 1/100 of the LD_{50} values. The fifth group received pesticide-free diet and was considered as control. Feeding lasted for thirty days. At the end of this period, rats were weighed, slaughtered and blood samples were immediately collected. Livers and kidneys of rats were dissected cleared from excess fat and weighed. Therefore, haematological, biochemical and histopathological studies could be carried out.

Blood samples

At the end of this experiment (30 days), blood samples were individually collected from each rat, immediately after slaughtering in dry clean centrifuge tubes and divided into two portions. The first portion contained heparin as anticoagulant (1–2 IU/ml) for haematological examination. The second portion was left to clot at room temperature for about 20 min and then centrifuged at 3000 r.p.m. for 15 minutes; the supernated serum samples were drown in dry clean-capped tubes and kept in deep freezer at –20 °C until conducting the biochemical analysis.

Haematological examination

Red blood cells (RBCs) and white blood cells (WBCs) were counted as described by Dacie and Lewis [9], while haemoglobin concentration (Hb) was measured according to Vankampen and Zijlstra [42].

Biochemical analysis

Activities of some enzymes and concentrations of certain biochemical parameters representing liver and kidney functions were determined in the rats blood sera colorimetrically as follows: Aspartate and alanine aminotransferase (AST & ALT) activities were determined according to the method of Schmidt and Schmidt [35], whereas acid and alkaline phosphatases (ACP & ALP) activities were determined as described by the method of Belfield and Goldberg [4] and Young and Friedman [46], successively. Total protein, albumin, glucose, cholesterol, uric acid and creatinine concentrations were determined according to the methods of [10, 11, 13, 19, 34, 40], respectively. All determinations were done by using U.S.A. Spectrophotometer Spekol 11.

Histopathological study

The influence of the tested pesticides on the histopathology of liver (the essential organ for drug metabolism) and kidney (the essential organ for drug excretion) was investigated. At the end of this experiment (30 days), liver and kidney from each sacrificed rat were removed, trimmed of excess fat and weighed (the relative weight of the organ equals the weight of the organ divided by the weight of whole rat body multiplied by 100). Then, the liver and kidney were fixed in 10% neutral formalin and prepared for histopathological examination according to Lillie and Fullmen [27].

Statistical analysis

Statistical analysis of toxicological experiment data was carried out according to Duncan's multiple range test [12].

RESULTS AND DISCUSSION

Effect on haematological parameters

Data recorded in Table 1 reveal that Vertimec caused a reduction in erythrocyte counts (RBCs), leukocyte counts (WBCs) and haemoglobin concentration. These effects were significantly more pronounced in Vertimec-treated rats at the high dose.

Table 1
Counts of red, white blood cells and haemoglobin concentration in blood of male albino rats fed on contaminated rations with tested toxicants for thirty successive days

Treatment	RBCs ($\times 10^6$/µl)		WBCs ($\times 10^3$/µl)		Haemoglobin (gm%)	
	count	% of control	count	% of control	conc.	% of control
1/10 LD_{50} Vertimec	3.31 ± 0.078^{c}	77.52	2.97 ± 0.089^{b}	74.62	14.229 ± 0.371^{b}	90.91
1/100 LD_{50} Vertimec	3.83 ± 0.075^{b}	89.69	3.72 ± 0.248^{a}	93.47	15.039 ± 0.074^{ab}	96.08
1/10 LD_{50} Agerin	3.88 ± 0.110^{b}	90.87	3.80 ± 0.189^{a}	95.48	15.665 ± 0.297^{a}	100.08
1/100 LD_{50} Agerin	3.95 ± 0.172^{ab}	92.51	3.98 ± 0.109^{a}	100.00	15.849 ± 0.546^{a}	101.25
Control	4.27 ± 0.129^{a}	100.00	3.98 ± 0.258^{a}	100.00	15.652 ± 0.104^{a}	100.00

Values in each column having the same superscript letter(s) were not significantly different ($p > 0.05$). Values are means of five determinations ± standard error (S.E.).

A significantly reduced amount of white blood cells could be indicative of immunosuppression [37]. The reduction in erythrocyte counts and consequently haemoglobin concentration may be attributed to more than one factor, i.e. the failure to supply the blood circulation with cells from haemohepatic tissues, since the liver has an important role in the regeneration of erythrocytes and the possible destructive effect on erythrocytes by the toxicants. The obtained results are in agreement with those found by many authors who stated that avermectins reduced erythrocyte, leukocyte counts and haemoglobin concentration in rabbits and rats [1, 2]. On the other hand, no haematological changes were found in the treated rats with Agerin. Similar results for *Bacillus thuringiensis* were observed by Tsai et al. [41].

Effect on liver function parameters

Liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver [3]. Therefore, liver can be used as an index for the toxicity of xenobiotics. Hence, the activities of some enzymes and levels of certain biochemical parameters representing liver function, i.e. AST, ALT, ACP, ALP, total protein, albumin, and cholesterol were determined in treated and untreated rats. Data in Tables 2 and 3 illustrate that Vertimec elevated the activity of AST and acid phosphatase (ACP) and decreased ALT activity, total protein, albumin, and glucose concentrations in serum of treated rats in a dose-dependent manner, whereas alkaline phosphatase (ALP) activity and cholesterol concentration remained unaltered. On the contrary, Agerin at both dose levels was innocuous for most chosen liver function indices with the exception of elevation of ACP activity and reduction of total protein concentration just at its high dose. The aforementioned findings are in coincidence with those of

Table 2
Serum aminotransferases and phosphatases activities of male albino rats fed on contaminated rations with tested toxicants for thirty successive days

Treatment	AST (U/L)		ALT (U/L)	
	activity	% of control	activity	% of control
1/10 LD_{50} Vertimec	67.65 ± 2.546[a]	194.23	15.95 ± 0.624[b]	68.16
1/100 LD_{50} Vertimec	60.02 ± 3.041[a]	172.32	20.63 ± 1.480[a]	88.16
1/10 LD_{50} Agerin	35.94 ± 6.044[b]	103.19	22.36 ± 0.300[a]	95.55
1/100 LD_{50} Agerin	34.93 ± 7.264[b]	100.29	23.57 ± 1.759[a]	100.73
Control	34.83 ± 1.598[b]	100.00	23.40 ± 0.794[a]	100.00
	Acid phosphatase (U/L)		Alk. phosphatase (IU/L)	
1/10 LD_{50} Vertimec	17.25 ± 3.464[a]	170.37	211.77 ± 1.482[a]	100.78
1/100 LD_{50} Vertimec	14.25 ± 0.433[ab]	140.74	207.97 ± 2.967[a]	98.97
1/10 LD_{50} Agerin	16.13 ± 1.082[a]	159.26	209.96 ± 0.958[a]	99.91
1/100 LD_{50} Agerin	13.88 ± 0.649[ab]	137.04	209.78 ± 0.941[a]	99.83
Control	10.13 ± 0.648[b]	100.00	210.14 ± 0.652[a]	100.00

Values in each column having the same superscript letter(s) were not significantly different ($p > 0.05$). Values are means of five determinations ± standard error (S.E.).

Table 3
Concentration of total protein, albumin, cholesterol and glucose in serum of male albino rats fed on contaminated rations with tested toxicants for thirty successive days

Treatment	Total protein (gm%)		Albumin (gm%)	
	level	% of control	level	% of control
1/10 LD_{50} Vertimec	7.60 ± 0.224[b]	86.27	5.63 ± 0.228[b]	84.16
1/100 LD_{50} Vertimec	8.09 ± 0.050[b]	91.83	6.68 ± 0.128[a]	99.85
1/10LD_{50} Agerin	7.61 ± 0.040[b]	86.38	6.63 ± 0.040[a]	99.10
1/100 LD_{50} Agerin	8.63 ± 0.256[a]	97.96	6.66 ± 0.109[a]	99.55
Control	8.81 ± 0.087[a]	100.00	6.69 ± 0.006[a]	100.00
	Cholesterol (gm%)		Glucose (gm%)	
1/10 LD_{50} Vertimec	34.17 ± 0.840[a]	98.19	60.79 ± 0.836[c]	82.71
1/100 LD_{50} Vertimec	34.80 ± 0.555[a]	100.00	65.38 ± 0.372[bc]	88.95
1/10 LD_{50} Agerin	33.12 ± 0.754[a]	95.17	66.13 ± 2.144[abc]	89.97
1/100 LD_{50} Agerin	33.75 ± 2.303[a]	96.98	70.09 ± 4.277[ab]	95.36
Control	34.80 ± 1.514[a]	100.00	73.50 ± 1.204[a]	100.00

Values in each column having the same superscript letter(s) were not significantly different ($p > 0.05$). Values are means of five determinations ± standard error (S.E.).

Hsu et al. [21] who reported that AST was elevated in abamectin-dosed rats in a dose-dependent manner; and Halkova et al. [17] who stated that *Bacillus thuringiensis* revealed no significant changes in biochemical parameters of sexually mature Wistar rats. AST and ALT are considered to be sensitive indicators of hepatocellular damage and within limits can provide a quantitative assessment of the degree of damage suffered by the liver [29]. Elevation of AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes [31] and is used to detect liver damage [26]. The alteration in serum levels of ALT may be indicative of internal organ damage especially of the liver [24]. The elevated ACP activity may be associated with the cell disintegration resulting from pesticide treatment, thus suggesting prenecrotic changes in the liver [33]. Qualitative and quantitative disturbance of protein synthesis is a consequence of impaired hepatic function [6]. Hypoalbuminemia (decreased albumin) is a liver disorder thought to be a consequence of decreased hepatic synthesis of albumin [5].

Effect on kidney function parameters

Results in Table 4 show that Vertimec increased uric acid and creatinine concentrations in the serum of treated rats in a dose-dependent manner. On the other hand, Agerin at both dose levels had no adverse effect on uric acid and creatinine concentrations in serum of treated rats. Uric acid and creatinine are useful in early detection of nephrotoxicity induced by exogenous compounds. These parameters are used as index of renal damage in living organisms [7]. Elevation of uric acid and creatinine concentration in serum of treated male albino rats may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules [18, 43].

Table 4
Concentration of uric acid and creatinine in serum of male albino rats fed on contaminated rations with tested toxicants for thirty successive days

Treatment	Uric acid (gm%)		Creatinine (gm%)	
	level	% of control	level	% of control
1/10 LD_{50} Vertimec	3.65 ± 0.181^a	177.18	1.23 ± 0.039^a	165.54
1/100 LD_{50} Vertimec	2.44 ± 0.173^b	118.45	1.07 ± 0.035^a	144.01
1/10 LD_{50} Agerin	2.15 ± 0.304^b	104.37	0.736 ± 0.093^b	99.06
1/100 LD_{50} Agerin	2.03 ± 0.353^b	98.54	0.723 ± 0.145^b	97.31
Control	2.06 ± 0.252^b	100.00	0.743 ± 0.108^b	100.00

Values in each column having the same superscript letter were not significantly different ($p > 0.05$).
Values are means of five determinations ± standard error (S.E.).

Histopathological changes

Relative weights of male albino rats organs

Results in Table 5 indicate that administration of Vertimec and Agerin at both dose levels resulted in a significant increase in the relative weight of treated male rat's liver in comparison with that of control rats. On the other hand, Vertimec and Agerin exhibited no significant alteration in the relative weight of treated male rat's kidney in comparison with that of control rats. Liver enlargement could be due to the accumulation of abnormal amounts of fat, predominately triglyceride, in the parenchymal cells. Triglyceride accumulation is a result of an imbalance between the rate of synthesis and the rate of release of triglyceride by the parenchymal cells into the systemic circulation [30].

Table 5
Weights of livers and kidneys of male albino rats fed on contaminated rations with tested toxicants for thirty successive days

Treatment	Post-treatment body weight (gm)	Liver		Kidney	
		weight (gm)	% weight	weight (gm)	% weight
1/10 LD_{50} Vertimec	226.66	7.61	3.36 ± 0.167[a]	1.63	0.717 ± 0.030[a]
1/100 LD_{50} Vertimec	202.70	6.77	3.35 ± 0.114[a]	1.42	0.710 ± 0.047[a]
1/10 LD_{50} Agerin	225.70	7.93	3.55 ± 0.177[a]	1.55	0.691 ± 0.016[a]
1/100 LD_{50} Agerin	205.00	6.95	3.38 ± 0.029[a]	1.34	0.654 ± 0.014[a]
Control	205.33	5.80	2.83 ± 0.087[b]	1.27	0.624 ± 0.027[a]

Values in each column having the same superscript letter were not significantly different ($p > 0.05$). Values are means of five replicates ± standard error (S.E.).

Liver and kidney histopathological examination

The normal structure of control liver and kidney of rats is shown in (Figs 1 and 4, respectively). Portal tract infiltration by lymphocytes and a focus of dysplasia with atypic cytology were observed in Vertimec-treated liver of rats at either dose levels (Fig. 2), while Agerin at the high dose solely caused mild portal tract infiltration by lymphocytes (Fig. 3). El-Banhawy et al. [14] found a remarkable abundance of lymphocytes infiltration in the liver tissues post-drug-administration, and postulated that such changes were a prominent response of body tissues facing any injurious impacts. Many reports had elucidated that hepatocellular damage could be correlated with the disturbed enzyme activities. In this respect, Rodwell [32] reported that liver tissue which is known to be rich in aminotransferases (AST & ALT) suffer markedly from their loss under many pathological conditions. Thus, the biochemical data obtained from this investigation support the assumption that Vertimec-treated rats suffered alteration in the activities of aminotransferases (AST & ALT). Concern-

ing the kidney, Vertimec at either dose levels induced interstitial nephritis in the kidney (Fig. 5), whereas Agerin caused hyaline globules inside the tubules with thickened membrane denoting nephritis (Fig. 6). Jencic et al. [22] demonstrated that the histological changes in rainbow trout organs showed a direct toxicity of abamectin since degenerative changes in brain and kidney and to a lesser extent in liver were detected.

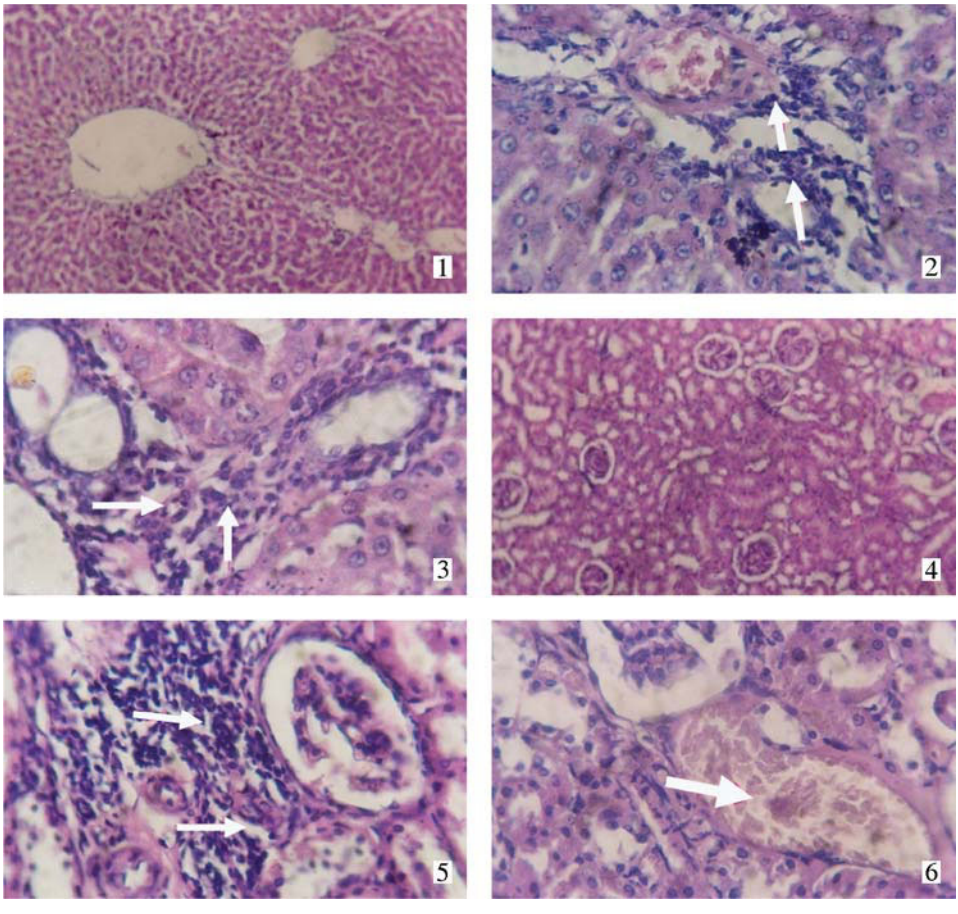

Fig. 1. A photomicrograph of a section of normal control male rat's liver (H & E × 10). *Fig. 2.* A photomicrograph of a section of Vertimec-treated male rat's liver at 1/10 LD_{50} level showing excess portal tract infiltration and a focus of dysplasia with cytological atypia (H & E × 40). *Fig. 3.* A photomicrograph of a section of Agerin-treated male rat's liver at 1/10 LD_{50} level showing mild-portal tract infiltration by lymphocytes (H & E × 40). *Fig. 4.* A photomicrograph of a section of normal control male rat's kidney (H & E × 10). *Fig. 5.* A photomicrograph of a section of Vertimec-treated male rat's kidney at 1/10 LD_{50} level showing interstitial nephritis ((H & E × 40). *Fig. 6.* A photomicrograph of a section of Agerin-treated male rat's kidney at 1/10 LD_{50} level showing hyaline globules inside the tubules with thickened membrane denoting nephritis (H & E × 40)

CONCLUSIONS

The findings of this study demonstrate that *Bacillus thuringiensis* used as commercial bio-pesticide is relatively safe. On the other hand, despite abamectin is a natural fermentation product of the soil-dwelling actinomycete *Streptomyces avermitilis,* it causes significant changes in RBCs, WBCs, haemoglobin, AST, ALT, ACP, total protein, albumin, glucose, uric acid, creatinine and liver relative weights in a dose-dependent manner. Likewise, pathological examination of abamectin-treated rats reveals abnormalities in the liver and kidney. Except for the above changes, there were no significant alterations found in ALP, cholesterol and kidney relative weights compared to the control group.

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