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Haematoxicity of a New Natural Insecticide "Spinosad" on Male Albino Rats

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ABSTRACT

Spinosad was administered orally to male albino rats in three different doses i.e., 0.02 mg a.i.kg^{-1} b.w. (ADI); 9 mg a.i.kg^{-1} b.w. (NOAEL) and 37.38 mg a.i.kg^{-1} b.w. (1/100LD$_{50}$) as single and 21 repetitive doses. After single oral treatment of the tested doses, no biologically meaningful differences in haematology results were recorded. While red blood cells (RBC's) and haemoglobin concentration (HGB) showed significant reduction after repetitive oral dose treatments with 9 mg a.i.kg^{-1} b.w. (NOAEL) and 37.38 mg a.i. kg^{-1} b.w. (1/100LD$_{50}$). Also, white blood cell count (WBC's), lymphocyte concentration (LYMF) and granulocyte concentration (GRAN) elevated significant increase ($p \leq 0.05$) after treatment with 0.02 mg a.i. kg^{-1} b.w. (ADI) and high significant increase ($p \leq 0.01$) after treatment with 9 mg a.i. kg^{-1} b.w. (NOAEL) or 37.38 mg a.i. kg^{-1} b.w. (1/100LD$_{50}$). There were no signs of toxicity noted during duration of the study, while remarkable lower body weight gains were observed.

Key Words: Haematology; Spinosad; Body weight gains; Rats

INTRODUCTION

Spinosyns, the new natural line of insect control products produced by fermentation of the actinomycete (*Saccharopolyspora spinosa*), were discovered in the 1980s (Thompson *et al*., 1997).

Spinosad is a mixture of two most active naturally occurring metabolites (spinosyns A & D) that is a new chemical class of insecticides to control a variety of insects and has an excellent environmental and mammalian toxicological profile (Crouse & Sparks, 1998; Sparks *et al*., 1998 & 1999; Thompson *et al*., 2000). Spinosad exhibits wide margins of safety to many beneficial insects and related organisms (Schoonover & Larson, 1995; Elzen *et al*., 2000) and is therefore considered a selective insecticide (Miles & Dutton, 2000).

Absolute selectivity, however, is difficult to achieve and most pesticides are toxic to a greater or lesser extent toward non-target organisms, including humans (Ernest & Patricia, 1997). Pesticides formulations are complex mixtures and the toxicity information on active ingredients alone is not sufficient to evaluate the risk of adverse health effects of commercial pesticides (Mansour & Mossa, 2005). The WHO (1991) emphasized the necessity of evaluating toxic hazard of the formulated pesticides. For this reasons, the objective of this study is to investigate the effect of acute and sub-acute doses of formulated Spinosad on the haematology and body weight gain of male albino rats.

MATERIALS AND METHODS

Insecticide. Tracers® (24% SC) is a commercial formulation containing spinosyns A and D (Dow AgroSciences Company). Spinosyn A is 2-[(6-deoxy-2, 3, 4-tri-O-methyl-alpha-L-mannopyranosyl) oxy)-13-[(5-dimethylamino) tetrahydro-6-methyl-2 H-pyran-2-yl) oxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a, 16b-tetradecahydro-14-methyl-1 H-as-indaceno (3, 2-d) oxacyclododecin-7, 15-dione. Spinosyn D is 2-((6-deoxy-2, 3, 4-tri-O-methyl-alpha-L-mannopyranosyl) oxy)-13-((5-(dimethylamino) tetrahydro-6-methyl-2 H-pyran-2-yl) oxy)-9-ethyl 2, 3, 3a, 5a, 5b, 6, 9, 10, 11, 12, 13, 14, 16a, 16b-tetradecahydro-4, 14-dimethyl-1 H-as-indaceno (3, 2-d) oxacyclododecin-7, 15-dione (Fig. 1).

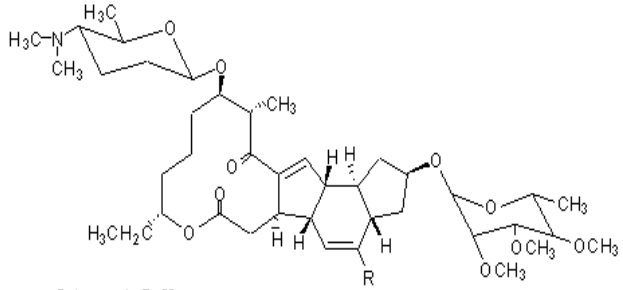
Spinosad has an acute oral LD$_{50}$ of 3738 mg kg^{-1} for male rats, No Observed Adverse Effect Level (NOAEL) for rats is 9 - 10 mg kg^{-1} b.w. daily and the ADI (JMPR) = 0.02 mg kg^{-1} b.w. (Anonymous, 2005).

Tested animals and dosing. Male albino rats (*Rattus norvegicus var. albus*), of 160 - 185 g were obtained from the animal breeding house of the National Research Centre (NRC), Dokki, Cairo, Egypt. The animals were acclimatized under laboratory conditions at room temperature of 23 ± 3.0^{o}C for one week. Food and water were provided *ad-libitum.*

Rats (10 rats/each dose) were given daily, via oral route, 21 repetitive doses equaled to 0.02 mg a.i.kg^{-1} b.w. (Acceptable Daily Intake for human, ADI), 9 mg a.i.kg^{-1} b.w. (No Observed Adverse Effect Level, NOAEL) and 37.38 mg a.i.kg^{-1} b.w. (1/100 LD$_{50}$) of spinosad using water as a solvent. Rats of control group were given the same volume of water (0.5 mL/rat) throughout the experimental durations.

Signs of toxicity and body weight gains. During the

Fig. 1. Chemical structure of Spinosad



experiment period, rats were observed for general appearance, behavior, symptoms of toxicity and mortality. Body weights were recorded weekly.

Blood collection. The blood samples were taken twice from retro-orbital venous plexus: once after 24 h (to test single dosing effect) and after 21 days following the beginning of the dosing (to test repetitive dosing effect). The blood was collected in small glass vials containing EDTA as an anticoagulant for haematological studies.

Measurement of blood constituents. These parameters have been assessed in respect of complete blood count (CBC), comprised Red Blood Cell Count (RBC's), Mean Cell Volume of RBC's (MCV), Red Cell Distribution Width (RDW), Haematocrit (HCT), Total Platelet Count (PLT), Mean Platelet Volume (MPV), White Blood Cell Count (WBC's), Haemoglobin Concentration (HGB), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), Lymphocyte Concentration (LYMF) and Granulocyte Concentration (GRAN). CBC was measured by Haematology analyzer (MEDONIC CA 620).

Statistical analysis. The experimental design was a factorial CRD (Complete Randomized Design) with ten replicates. Statistical analysis of data collected was carried out using a computer program (Cohort Software, 1986).

RESULTS

The present study was carried out to determine some toxic effects of a formulated form of spinosad, administered orally to male rats at two dosing levels: single and repetitive treatments.

Signs of toxicity. No obvious signs of toxicity were noted during the experimental duration in behavioral activity or external appearance in any of the treated rats. Furthermore, no mortality was occurred (data are not tabulated).

Haematological parameters. Data of haematological parameters are shown in Table I and II, for single and repetitive treatments, respectively. Results in Table I indicate no biologically meaningful differences in haematology results after single oral dose in any of the treatments. On the other hand, after repetitive oral dose treatments (Table II), red blood cells (RBC's) and haemoglobin concentration (HGB) showed significant reduction as compared with the un-treated control group. The decrease in RBC's count was 5.45% and 6.94% after repetitive oral dose treatments with 9 mg a.i.kg^{-1} b.w. (NOAEL) and 37.38 mg a.i. kg^{-1} b.w. (1/100 LD_{50}), respectively and the decrease in HGB was 7.46%, 7.71% and 5.14% after repetitive oral dose treatments with 0.02 mg a.i.kg^{-1} b.w. (ADI), 9 mg a.i.kg^{-1} b.w. (NOAEL) and 37.38 mg a.i. kg^{-1} b.w. (1/100 LD_{50}), respectively. In contrast, white blood cell (WBC's), lymphocyte concentration (LYMF) and granulocyte concentration (GRAN) recorded significant increases ($p \leq 0.05$) after treatment with 0.02 mg a.i. kg^{-1} b.w. (ADI) and accounted to 24.83%, 15.80% and 30.35%, respectively for the above-mentioned parameters. Similarly, highly significant increases ($p \leq 0.01$) were recorded after repetitive oral dose either with 9 mg a.i. kg^{-1} b.w. (NOAEL) or with 37.38 mg a.i. kg^{-1} b.w. (1/100 LD_{50}) and accounted to 53.17%, 56.09%, 39.29% and 71.90, 82.39, 51.10%, respectively, for the three above-mentioned parameters.

Body weight gains. Results in Table III show the effect of treatment with spinosad on rat's body weight gain during the experimental duration. Generally, there was an increase in body weight with time elapsed. After 3 weeks of treatment, the body weight gain, of rats given 9.0 and 37.38 mg a.i kg^{-1}b.w., was decreased significantly ($p \leq 0.01$) comparing with control. The percentage of weekly body weight gain of control was 6.25, compared to 5.31, 3.05 and 1.00 for the treatments with ADI, NOAEL and 1/100 LD_{50}, respectively. On the other hand, the % of weekly body weight change with time elapsed is demonstrated in Fig. 2a, which indicates that the change was accounted to 2.05%, 0.38% and 0.54% after 1st, 2nd and 3rd week of treatment with 1/100 LD_{50}. In comparison, the changes were respectively 4.60%, 4.91% and 5.65% for the treatment of ADI dose. It appears that the ADI treatment, like control, possessed positive slope values, while the other two tested doses (9.0 & 37.38 mg kg^{-1}b.w.) possessed negative slope values as shown from the straight-line equations "y = mx + b" (Fig. 2b).

DISCUSSION

Spinosad is primarily a stomach poison with some contact activity and is particularly active against Lepidoptera and Diptera. It represents a new class of insecticides acting by a neurotoxin with a novel mode of action and act as an agonist at the post-synaptic cholinergic ion channels and GABA-gated ion channels (Salgado, 1998; Salgado *et al*., 1998; Thompson *et al*., 2000). For this reason, signs of toxicity were recorded and results showed that there were no treatment-related clinical signs during the duration of study at any of the tested doses.

A complete blood count provides detailed information about three types of blood cells: red blood cells (RBC's), white blood cells (WBC's) and platelets. These blood cells

Table I. Haematological effects of Spinosad in rats administered single oral doses

		Doses of Spinosad (mg a.i. kg^{-1} b.w.)					
Parameters	**Control**	**0.02 (ADI)**	**% of Change**	**9.0 (NOAEL)**	**% of Change**	**37.38 (1/100 LD_{50})**	**% of Change**
RBC's ($10^6/mm^3$)	8.89±0.10	8.99±0.18	1.12	8.86±0.13	-0.34	8.79±0.18	-1.12
MCV (μm^3)	50.60±1.32	49.67±2.01	-1.84	50.24±1.22	-0.71	50.19±0.24	-0.81
RDW %	14.96±1.02	14.88±1.02	-0.53	15.00±1.02	0.27	14.79±1.02	-1.14
HCT %	45.00±0.55	44.66±0.28	-0.76	44.52±0.12	-1.07	44.12±0.32	-1.96
PLT ($10^3/mm^3$)	443.6±44.1	438.0±46.1	-1.26	453.6±38.4	2.25	423.6±39.1	-4.51
MPV (μm^3)	6.46±0.23	6.40±0.14	-0.93	6.38±0.05	-1.24	6.18±0.36	-4.33
WBC's ($10^3/mm^3$)	15.88±1.63	15.98±1.02	0.63	16.14±1.88	1.64	16.28±1.84	2.52
HGB (g/dl)	15.16±0.23	15.08±0.11	-0.53	14.97±0.37	-1.25	14.56±0.44	-3.96
MCH (pg)	17.05±0.05	16.77±0.56	-1.64	16.89±1.01	-0.94	16.56±1.14	-2.87
MCHC (g/dl)	33.68±1.05	33.76±0.85	0.24	33.62±1.07	-0.18	33.00±1.22	-2.02
LYMF ($10^3/mm^3$)	8.86±0.34	8.90±0.12	0.45	8.99±0.45	1.47	9.11±0.14	2.82
GRAN ($10^3/mm^3$)	5.82±0.22	5.78±0.14	-0.69	5.88±0.43	1.03	6.00±0.38	3.09

Values are means ± S.D; statistical difference from the control: *significant at $P \leq 0.05$ and**highly significant at $P \leq 0.01$.
% of change = [(treatment – control)/control] x 100

Table II. Haematological effects of Spinosad in rats administered 21 repetitive oral doses

		Doses of Spinosad (mg a.i. kg^{-1} b.w.)					
Parameters	**Control**	**0.02 (ADI)**	**% of Change**	**9.0 (NOAEL)**	**% of Change**	**37.38 (1/100 LD_{50})**	**% of Change**
RBC's ($10^6/mm^3$)	9.36±0.15	8.99±0.11	-3.95	8.85±0.17*	-5.45	8.71±0.19*	-6.94
MCV (μm^3)	47.20±1.30	45.49±1.02	-3.62	46.86±0.92	-0.72	49.36±2.59	4.58
RDW %	15.76±1.02	17.50±1.30	11.04	17.16±1.06	8.88	16.16±0.75	2.54
HCT %	44.06±0.58	40.90±1.08	-7.17	41.50±0.43	-5.81	43.06±3.16	-2.27
PLT ($10^3/mm^3$)	433.3±42.3	385.6±41.1	-11.01	463.0±40.7	6.85	417.3±39.2	-3.69
MPV (μm^3)	6.46±0.25	6.26±0.05	-3.10	6.56±0.23	1.55	6.53±0.20	1.08
WBC's ($10^3/mm^3$)	16.23±1.34	20.26±1.89*	24.83	24.86±0.9**	53.17	27.90±1.37**	71.90
HGB (g/dl)	15.56±0.37	14.4±0.30*	-7.46	14.36±0.15*	-7.71	14.76±0.80*	-5.14
MCH (pg)	16.70±0.70	16.01±0.28	-4.13	16.26±0.49	-2.63	16.96±0.60	1.56
MCHC (g/dl)	35.36±0.60	35.23±0.20	-0.37	34.70±0.75	-1.87	34.36±0.61	-2.83
LYMF ($10^3/mm^3$)	8.86±0.47	10.26±0.49*	15.80	13.83±1.18**	56.09	16.16±1.50**	82.39
GRAN ($10^3/mm^3$)	5.93±0.43	7.73±1.17*	30.35	8.26±0.30**	39.29	8.96±0.55**	51.10

Values are means ± S.D; statistical difference from the control:*significant at $P \leq 0.05$ and**highly significant at $P \leq 0.01$.
% of change = [(treatment – control)/control] x 100

Table III. Body weight gain in male rats administered 21 repetitive oral doses of Spinosad

Doses (mg kg^{-1} b.w.)	**Body weight (g)**				
	Initial b.w.	**1st week b.w.**	**2nd week b.w.**	**3rd week b.w.**	**% of weekly b.w. gain**
0	171.8±6.6	181.0±8.4	191.0±10.9	204.0±6.7	6.25
0.02	169.4±4.6	177.2±11.0	185.9±6.4	196.4±4.5	5.31
9.0	175.8±3.9	181.7±10.3	186.3±6.8	191.9±4.3**	3.05
37.38	180.1±4.2	183.8±7.3	184.5±7.6	185.5±7.9**	1.00

Values are means ± S.D; statistical difference from the control:*significant at $P \leq 0.05$ and**highly significant at $P \leq 0.01$.
% of weekly body weight gain = [(final b.w - initial b.w.)/ (initial b.w. x no. of weeks)] x 100

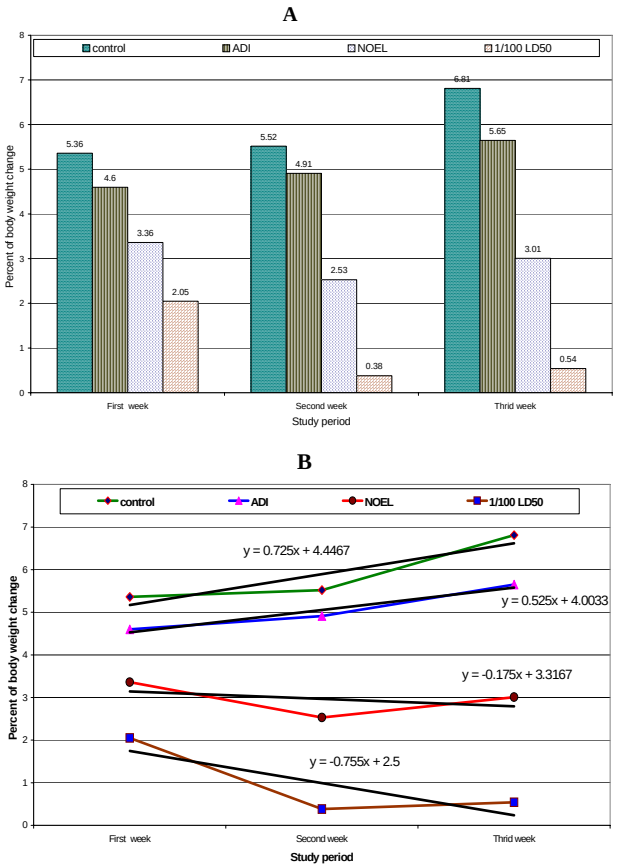
are made in the bone marrow. Furthermore, haematological characteristics have been widely used in the diagnosis of variety of diseases and pathologies induced by industrial compounds, drugs, dyes, heavy metals, pesticides and several others (Morgan & Stockdale, 1980; Ali *et al*., 1988; Ali & Shakoori, 1988; Mossa, 2004; Mansour & Mossa, 2005).

Red blood cells (known as erythrocytes) are very important for the transport of oxygen from the lungs to the tissues and haemoglobin concentration is directly correlated with RBC's count. This is due to the synergistic link among these blood parameters in all vertebrates (El-Bakary *et al.,* 1995). This close correlation between erythrocyte count and haemoglobin concentration was also reported for other vertebrates including man (Harris, 1972). In our study, reduction in HGB content may be due to increased rate of breakdown of red cells and/or reduction in the rate of formation of RBC's. Recorded low RBC's in the treated groups supported this premise. Shakoori *et al.* (1990) suggested that the decrease in RBC's is either indicative of excessive damage to erythrocytes or inhibition of erythrocyte formation in rabbits. Moreover, the hepatic heme biosynthesis has already been reported to be affected by insecticidal exposure, which also contributes to decreased HGB and RBC's count (Taljaard *et al.*, 1972). Also, many laboratories have reported the induction of anemia with experimental insecticidal exposure of animals (Ali & Shakoori, 1990).

Our findings are in agreement with the results reported by Yano *et al.* (2002), who found that male rats given 0.2%

Fig. 2. Percent of change in weekly body weight in male rats received 21 repetitive oral doses of Spinosad

N.B: % of weekly body weight change = [(b.w. at end of a week – b.w. at beginning of the week)/b.w. at beginning of the week] x 100 [Based on data of Table III]



spinosad for 13 weeks had significant decreases in HGB concentration (60%) and RBC's count (11%) relative to control. The authors referred the observed anemia to the decrease in HGB and RBC's count. Stebbins *et al.* (2002) reported that erythrocytic parameters (RBC's count & hemoglobin concentration) were decreased approximately 10 - 20% in male mice given 0.036% spinosad after 3 and 12 months. Shakoori *et al.* (1990) found decrease in erythrocytic, leukocytic counts, PCV and HGB content in the blood of bifenthrin-treated rabbits. Also, El-Sahhaf (1995), Yousef *et al.* (1999), Mossa (2004) and Mansour and Mossa (2005) found that the direct effect of pesticides is a reduction in the total number of erythrocytes, PCV and HGB content. It was thought that these changes were due to an increased rate of breakdown of red cells and/or the toxic effect of pesticides on bone-marrow.

White blood cells (WBC's) formed in the bone marrow either enters the blood or migrates to key organs such as the spleen, lymph nodes, or gut. The increased number of leukocytes can occur abnormally as a result of an infection, cancer, or toxic chemical. Such increase of WBC's may be due to the activation of the animal's defense mechanism and the immune system. Also, several chemical compounds including insecticides, generate auto-antibodies due to their interference with immune system, which could be the reason for bone-marrow injury due to exposure to toxic pollutants.

The observed effects of spinosad insecticide, which represented by increase of white blood cells counts (WBC's), lymphocyte concentration (LYMF) and granulocyte concentration (GRAN) in the blood of the treated rats are generally in agreement with the results of several investigations. Yano *et al.* (2002) reported that after rat exposure to spinosad, white blood cell count of females from the 0.1% group was 39% higher than the controls after 18 months and noted that this difference was likely related to inflammation of the lung and thyroid gland in these rats. Stebbins *et al.* (2002) found that WBC's counts of male mice and females given 0.036% spinosad and females given 0.024% spinosad, were 2 - 2.5 times higher than the controls after 12 months and noted that the higher WBC's counts were likely related to inflammation of the stomach observed in these mice.

Results of other studies done by Shakoori *et al.* (1990) on rabbits, Fujiani *et al.* (1997) on rats, Nuberger *et al.* (1998) on human, Mossa (2004) and Mansour and Mossa (2005) in rats showed that treatment with different pesticides markedly elevated the animal's defense mechanism and immune system.

Our findings revealed significant increase in body weight gain, lower than that of control, after 3 weeks in the rat groups treated with 9.0 and 37.38 mg a.i kg^{-1}b.w. of spinosad. However, Breslin *et al.* (2000) on rabbits given 50 mg/kg/day spinosad and Stebbins *et al*. (2002) on male mice treated with 109.7 mg/kg/day spinosad have observed significant decrease in body weight gain. In this respect, it has to mention that exposure periods, dose level and test species have to be considered.

In conclusion, spinosad as a formulated form "Tracers®; 24% SC" under the present experimental conditions seems to be toxic to rat haematological parameters especially RBC's, HGB, WBC's, LYMF and GRAN and markedly lowered the rate of body weight gains compared to un-treated animals. Such results may highlight the necessity of evaluating the toxic hazards of spinosad at very low doses.

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