



Original Research Article

Toxicity evaluation of spinosad on male wistar rats

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***Adebiyi, Opeymi. E.¹,
Fadina Olubunmi.O.²,
Fayinminnu Olajumoke. O.²,
Idowu-Agida, Oladayo.O¹,
Oladigbolu, Abiola.A¹,
Oladosu Bolanle. O¹
And
Dixon Henry.A¹.**

¹National Horticultural Research
Institute, Jericho Ibadan

²Department of Crop Protection
and Environmental Biology,
University of Ibadan

*Corresponding Author Email:
adebiyiope@gmail.com

This study was carried out at the animal experimental house, department of Animal Science, University of Ibadan. Twenty rats weighing between 150-165g were used for the experiment for a period of five weeks, laid out in a Completely Randomized Design (CRD) with five groups of 4 rats per treatment including control. The rats were fed with Spinosad preserved maize incorporated feed. The doses of 0.5mg/kg, 1mg/kg (recommended), 1.5 and 2mg/kg were administered once daily for 3 weeks. Toxicological effects were studied and were analysed using descriptive statistics at $p \geq 0.05$. The results showed decrease in body weights in rats in accordance to increase in doses. Gross pathological cyst and irregularities were observed in all the livers while the kidneys showed no significant changes to spinosad. Some haematological parameters such as packed cell volume (PCV), Haemoglobin count (Hgb), Red blood cell (RBC), White blood cell (WBC), revealed no significant differences except the monocytes. The serum biochemical tests such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Albumin (Alb) and Globulin (Gb) revealed significant differences in values especially among rats treated. Comparism between the recommended (1.00mg/kg) and various levels of overdose (1.5 and 2.00mg/kg) has significant differences. Spinosad as a formulated form "Tracers®" under the present experimental conditions seems to be toxic to liver at a particular dosage by varied changes in cyst observed and in the levels of the heamatology, biochemical parameters and histopathology changes as a result of different concentrations in the treated groups relative to the control group. The overdoses of spinosad were found out to negatively affect liver and impaired its function; hence negatively impact the health of the animals. Abuse, misuse or overdoses of spinosad (bio-insecticide) should be discouraged.

Key words: Bio-insecticide, histological, organs, spinosad, serum biochemistry, wistar rats.

INTRODUCTION

Pests cause unquantifiable damage and threaten food supply, property, health, and the livelihood of growers. A lot of pests have a remarkable ability to adapt to environmental pressures, to changes in cropping systems, cultural practices and climate, and to tolerate or resist management strategies including the use of pesticides. The use of synthetic pesticides has greatly been discouraged because of its ability to bio-accumulate, bio-magnify its

residence period and human toxicity hence, the encouragement on the development and use of bio-pesticides. The consequences of synthetic pesticides use in developing countries are fatal (PAN, 2000). Although pesticides may be selectively toxic to these forms of life, they may still be toxic to man if food contaminated by them is ingested (Ademoroti, 1996). Spinosad which consist of spinosyn A and D is derived from a naturally occurring, soil-

dwelling bacterium, *Saccharopolyspora spinosa*. Spinosyns are neurotoxins that activate postsynaptic nicotinic acetylcholine and gamma-aminobutyric acid receptors and cause rapid excitation of the insect nervous system and ultimately exhaustion and death of the targets. Spinosad, a reduced-risk commercial insecticide has been labeled for use on over 250 crops in more than 50 countries (Mertz and Yao, 1990; Thompson et al., 2000). Spinosad has low mammalian toxicity and degrades quickly when exposed to sunlight (Thompson et al., 2000), but it was relatively stable in stored-grain (Fang et al., 2002; Flinn et al., 2004). These been properties make it an ideal product for use in stored grain. Laboratory and field tests on stored wheat showed that spinosad at 1 mg/kg active ingredient (a.i.) of grain was effective against several insect pests including the lesser grain borer *Rhyzoperthadominica* (F.), rusty grain beetles *Cryptolestes ferrugineus* (Stephens), and Indian meal moth *Plodiainterpunctella* (Hübner) (Fang et al., 2002a, 2002b; Flinn et al., 2004; Huang et al., 2004).

In 2005, the United States Environmental Protection Agency registered spinosad at 1 mg/kg as a grain protectant on commodities including wheat, corn, rice, millets, oats, sorghum, and barley (Bruggink, 2005).

Synthetic chemicals are hazardous to human, animals and the environment such as their effects on micro-organisms, pollinators, earthworm etc. Therefore, there is a need to test the natural bio-pesticide (spinosad) for use in storage. The objective of this study was therefore to investigate the haematological, serum biochemical and histopathological effects caused by exposure to sub-chronic concentrations of Spinosad on wistar rats.

MATERIALS AND METHODS

This study was carried out at the department of Animal science, Clinical pathology laboratory and Histopathology laboratory at department of Veterinary Pathology, University of Ibadan, Nigeria. The rats used for this study was gotten from department of Veterinary, University of Ibadan, untreated maize was gotten from Institute of Agric Research and Training, MOORE plantation, Ibadan and the spinosad used was gotten from SARO agro chemical Ibadan.

A total of 20 male albino (wistar) rats were used. The rats were divided into five groups with each rat occupying a unit/cell of the cage, making it five treatments replicated four times and the rats were acclimatized for two weeks before commencement of treatments. Feed and water were given to the animals during acclimatization and treatments periods ad libitum. The Spinosad treatments were 0mg/kg (control), 0.5mg/kg, 1mg/kg (recommended), 1.5mg/kg and 2mg/kg. All the treatments were administered daily as it was included in the feed given for 21 days. On the termination of the experiment, all the rats were euthanized by cervical dislocation and organs of interest were harvested. These include the liver and kidney for histopathological examination. Also 2mls of blood samples was collected through the ocular orbit into vacuum

container EDTAK3 tubes by heparinized capillary tubes, according to Schalm, (1986). The blood samples for some serum biochemical analysis were collected by heparinized capillary tubes into lithium heparinized tubes. The experiment was laid out in complete randomized design (CRD) and data were analyzed with one way analysis of variance (ANOVA), means were compared at 95% confidence level (P 0.05) by Least Significant difference (LSD). Results were presented as mean \pm standard error of the means (SEM).

RESULTS AND DISCUSSION

Spinosad on some haematological parameters of male wistar rats

Results from this study revealed significant difference in some haematological parameter (Monocyte). No significant difference was recorded in the packed cell volume from rats treated with 0.5mg/kg, 1mg/kg, 1.5mg/kg, 2mg/kg and control (Table 1). However, rats dosed with 0.5mg/kg recorded highest mean value for packed cell volumes while the lowest mean value was obtained in treatment 2mg/kg. The haemoglobin recorded from treatment 0.5mg/kg, 1mg/kg, 1.5mg/kg, 2mg/kg and control also showed no significant difference, but the highest mean value for haemoglobin was recorded from the treatment with 0.5mg/kg while lowest was from 2mg/kg. In Pack cell volume and haemoglobin, no significant difference was observed in the white blood cell of all the rats. Although, rats without spinosad (control) had highest means value for white blood cell while treatment 1.5mg/kg recorded lowest mean value (Table 1).

The result of monocytes showed that there was a significant difference between the rats treated with 1mg/kg and treatment with 1.5mg/kg, rats treated with 1.5mg/kg recorded significantly higher mean value for the monocytes while those with 1mg/kg recorded the lowest mean value. There was no significant difference amongst all treatments and control in the other haematological parameters (red blood cell, neutrophils and lymphocytes). The neutrophils had highest mean value in animals treated with 1mg/kg and lowest in the 2 mg/kg (Table 1).

Spinosad levels on some serum biochemical parameters of wistar rats.

There was significant difference in Aspartate amino transferase, (AST) of animals treated with 1mg/kg and 1.5mg/kg, although no significance difference was observed amongst the other treatments and the control. However, the rats treated with 1mg/kg recorded highest means value while lowest was from control. There were no significant differences in Alkaline Phosphate (ALP), total bilirubins and eosin in all the treatments compared with the control but 1.5mg/kg was highest in mean value and lowest in the control with respect to Alkaline Phosphate. In Total

Table 1. Effects of varying levels of Spinosad on haematological parameters of wistar rat

Dosage (mg/kg)	PCV (%)	Hb (Gg/dl)	WBC (cells/mm ³)	RBC (X10/L)	NEUT (%)	LYMPH (%)	MONO (%)
0	43.00±2.12a	14.33±0.71a	6750.0±2015.56a	7.28±0.54a	37.00±4.14a	55.25±3.71a	2.75±0.63ab
0.5	44.00±2.35a	14.67±0.78a	5800.0±1570.70a	7.23±0.24a	34.75±4.73a	61.25±4.91a	2.50±0.50ab
1	39.75±2.02a	13.25±0.67a	4037.5±1397.52a	7.05±0.19a	39.75±2.32a	55.00±1.96a	2.00±0.41a
1.5	38.25±2.95a	12.75±0.98a	6187.5±761.13a	6.71±0.48a	38.00±4.02a	55.50±3.48a	4.25±0.75b
2	37.50±0.50a	12.50±0.17a	6075.0±1565.05a	6.88±0.08a	30.25±4.03a	63.00±3.63a	3.50±0.29ab

Means within the same column followed by the same letter(s) are not significantly different at P < 0.05 using DMRT, PCV= Packed Cell Volume, HB= Haemoglobin, WBC= White Blood Cell counts, RBC = Red blood cell count, NEUT= Neutrophiles, LYMPH= Lymphocytes, MONO = Monocytes

Table 2. Effects of varying levels of Spinosad on Serum Biochemical parameters of wistar rats

Dosage(mg/kg)	TB	EO	ALP	AST	ALT	TP	ALB	GLB
0	3.30±1.95a	2.50±0.87a	104.25±9.36a	47.87±7.60a	-4.40±0.69a	7.74±0.62a	3.92±0.68b	3.82±0.48a
0.5	4.78±1.95a	1.50±0.65a	110.00±3.34a	60.80±3.31ab	-2.12±0.69a	9.52±1.18a	1.82±0.74a	7.69±0.72b
1	5.03±1.71a	3.25±0.85a	114.50±1.66a	78.36±5.07b	-2.44±0.43a	7.83±0.11a	2.46±0.44ab	5.37±10.54a
1.5	2.58±1.46a	2.25±0.48a	117.75±2.32a	48.92±4.17a	5.84±4.42b	8.26±0.35a	2.89±0.68ab	5.37±1.01a
2	2.33±1.62a	3.25±0.48a	114.50±4.66a	59.06±9.36ab	13.32±0.27c	7.75±0.22a	3.20±0.52ab	4.55±0.44a

Means within the same column followed by the same letter(s) are not significantly different at P < 0.05 using DMRT, AST = Aspartate amino transferase, TB = Total bilirubin, EO = Eosine, ALP = Alkaline Phosphate, ALT = Alanine amino transferase, TP = Total protein, ALB= Albumin, GLB = Globulin

bilirubin, 1mg/kg had highest mean value while 2mg/kg recorded lowest mean value, and in Eosin the highest mean value was observed in animals treated with 2mg/kg and lowest in 0.5mg/kg. (Table 2).

The result showed that there was significant difference in alanine amino transferase (ALT) between treatment 1mg/kg, 1.5mg/kg and 2mg/kg, highest in mean value was observed 2mg/kg while control recorded lowest mean value. The trend in total protein estimation showed no significant difference between all treatment and control while the highest mean value was observed in animals treated with 0.5mg/kg and lowest in control. There was significant difference in the control and treatments with 0.5mg/kg for albumin where control had the highest mean value and treatment with 0.5mg/kg had the lowest mean value (Table 2).

All rats treated with Spinosad showed a marked decrease in body weight while untreated that is control continues to gain weight during the period of treatments and the rats showed some signs of pesticide poisoning and this is in contrast with the work of Mansour et al. (2007) who reported that there were no signs of toxicity noted in rats treated with spinosad.

The trend in lymphocytes revealed highest mean value in animals treated with 2mg/kg and lowest in 1mg/kg as shown in (Table 1). The observed effects of spinosad insecticide, which was in haematological parameters of treated rats are generally in agreement with the results of several investigations. Yano et al. (2002) reported that after rat exposure to spinosad, male rats given 0.2% spinosad for 13 weeks had significant decreases in HGB concentration (60%) and RBC's count (11%) relative to control and noted

that this difference was likely related to inflammation of the lung and thyroid gland in these rats.

The reduction of serum levels of protein for all the rats dosed with the Spinosad at all dosages of treatment correlates with the findings of Das and Mukherjee (2000), also reported that toxicants may cause stress-mediated mobilization of protein to cope with the detrimental condition so imposed. The protein mobilized is one of the strategies employed to meet the energy required to sustain increased physical activity, biotransformation and excretion of the toxicants.

Aminotransferases (ALT and AST) are produced in the liver and are good markers of damage to liver cells but not necessarily the severity of the damage as reported by (Olav et al., 2007; Rej, 1989). The reduced levels of AST, ALB and ALT in Spinosad treated groups could be as a result of suppression of production by the liver this agree with Obaineh and Mathew, (2009) says they are normally present at low levels in the blood so if the liver cells are damaged, it would be expected that some of the enzymes leak into the blood and increase the levels. Decrease albumin may be due primarily to reduction in synthesis by the liver and secondarily to reduced protein intake which further confirms hepatic damage and agree with the works of (Luskova et al., 2002; Jyotsna et al., 2003) as observed in control and 0.5mg/kg in the study. Decreased albumin observed in this work revealed that Spinosad administration may be attributed to hepatic dysfunction in the overdose. In contrast to serum enzymes, albumin apart from being a useful indicator of the integrity of glomerular membrane is also an important indicator in determining the severity of disease as also stated by (Lawrence and

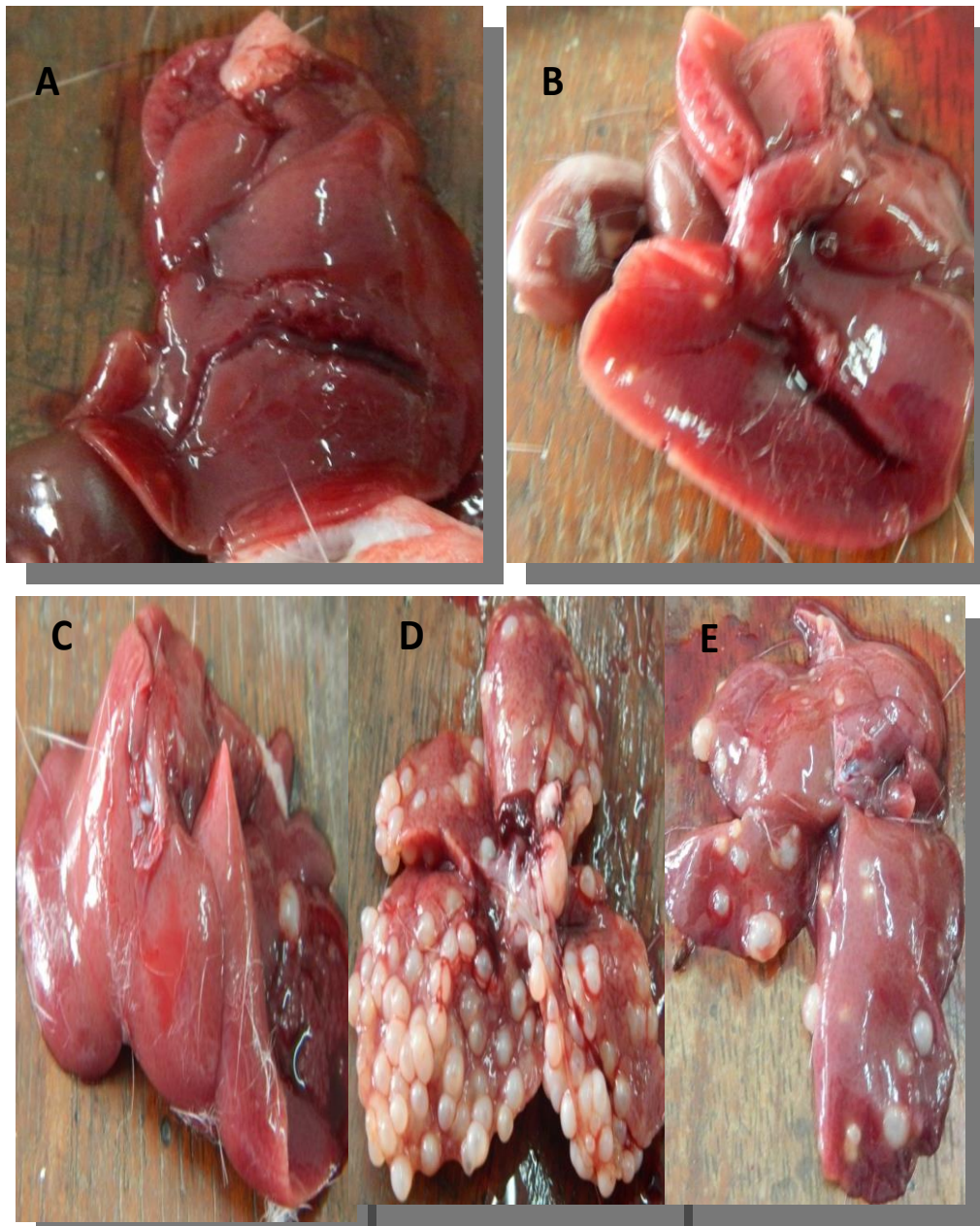


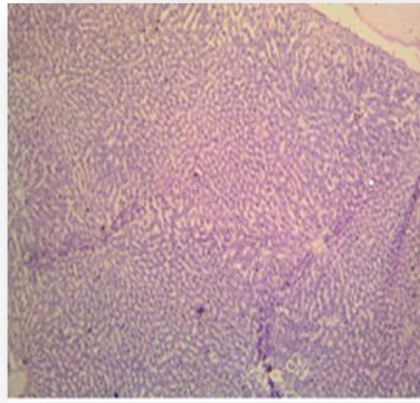
Plate 1A-E: Various levels of cyst observed in livers of wistar rats treated with spinosad. A= Control, B=0.5mg/kg C=1mg/kg D=1.5mg/kg E=2mg/kg

Amadeo, 1989; Mukut et al., 2001). In the histopathological evaluation of the liver and kidney of rats treated with Spinosad, the pattern of injury and the liver lesion can be described as a "Toxic Liver" (Plate 1A-E) which varies with the doses administered and this is in contrast with the work of McCormack, (2011). In the various treatments, the only observable change is an increased vascular congestion ranging from mild to moderate (Plate 2). The spinosad does not have a structural change on the tubules and other anatomic structures in the kidney. The toxicity effects of spinosad is acute and sub-acute as the clinical symptoms manifested earlier and test animal recovered before the

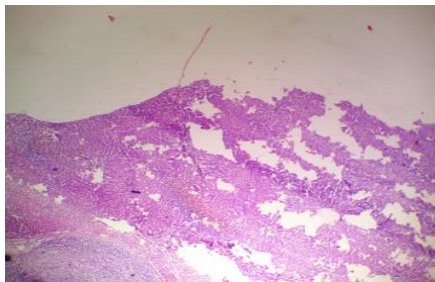
third week of treatments. This is in contrast with the work of McCormack, (2011).

Feed intake and weight gain.

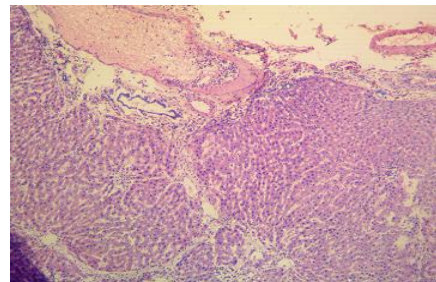
It was observed that there were no significant differences in the average feed intake of experimental animals dosed with 0mg/kg, 0.5mg/kg, 1mg/kg and 2mg/kg across the three weeks of treatments except for treatment 1.5mg/kg where there was a significant difference at week two where they had a reduced feed intake (Figure 1). There was also no significant difference in the average weight gain of the



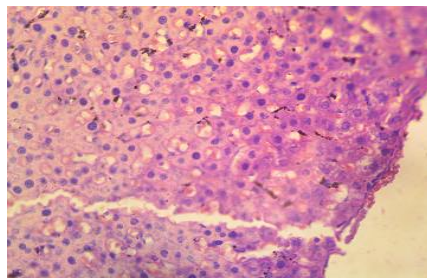
0 mg/kg (control)



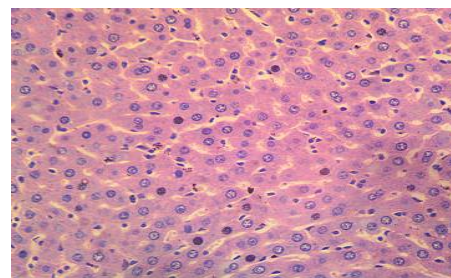
0.5mg/kg



1mg/kg



1.5mg/kg



2mg/kg

Plate 2: Photomicrograph of histopathology of livers of wistar rats treated with spinosad

Mag: (x400) Microscope Type : Light microscope

Key: Liver 1 (Control) - Closely packed with hepatic cell. No visible lesion.

Liver 2 (0.5mg/kg) - No lesion observed

Liver 3 (1mg/kg) - Moderate vacuolation of hepatocytes (diffuse).

Liver 4 (1.5mg/kg) - There is moderate, diffuse vacuolation (presence of clear vacuoles in the cytoplasm of the liver cells). It is a degenerate change or indication of fatty liver.

Liver 5 (2mg/kg) - Severe oedema, vacuolation, tissue hyperemia and individualization of hepatocytes.

treated animals except for animals dosed with 2mg/kg and 1.5mg/kg that had the lowest weight respectively (Figure

2) and this is in conformity with the work of (Ademoroti, 1996).

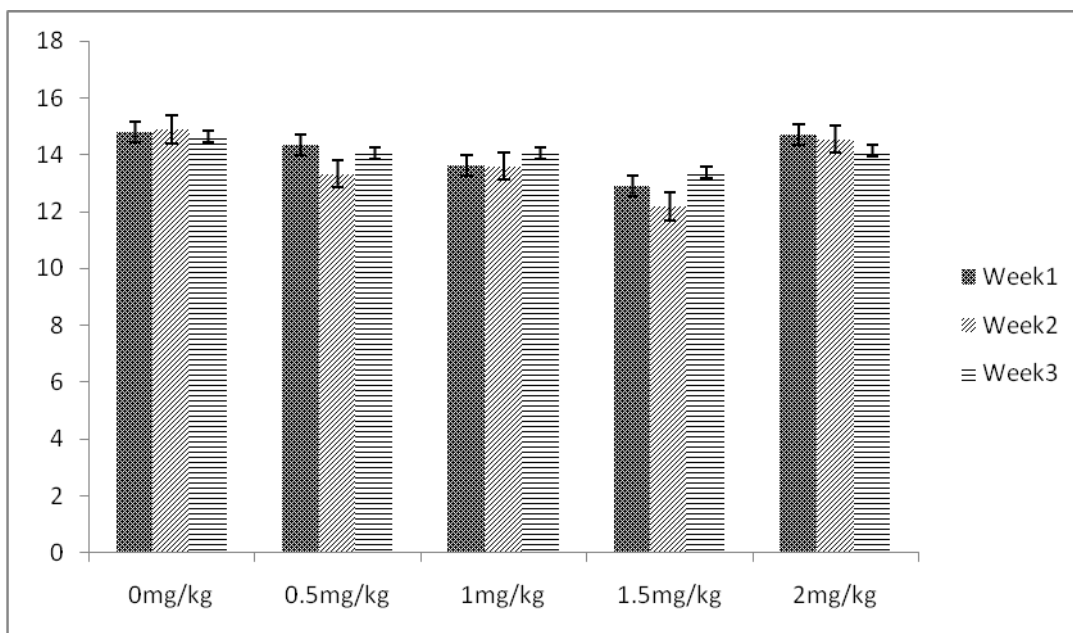


Figure 1: Average feed intake of wistar rats treated with varying doses of spinosad

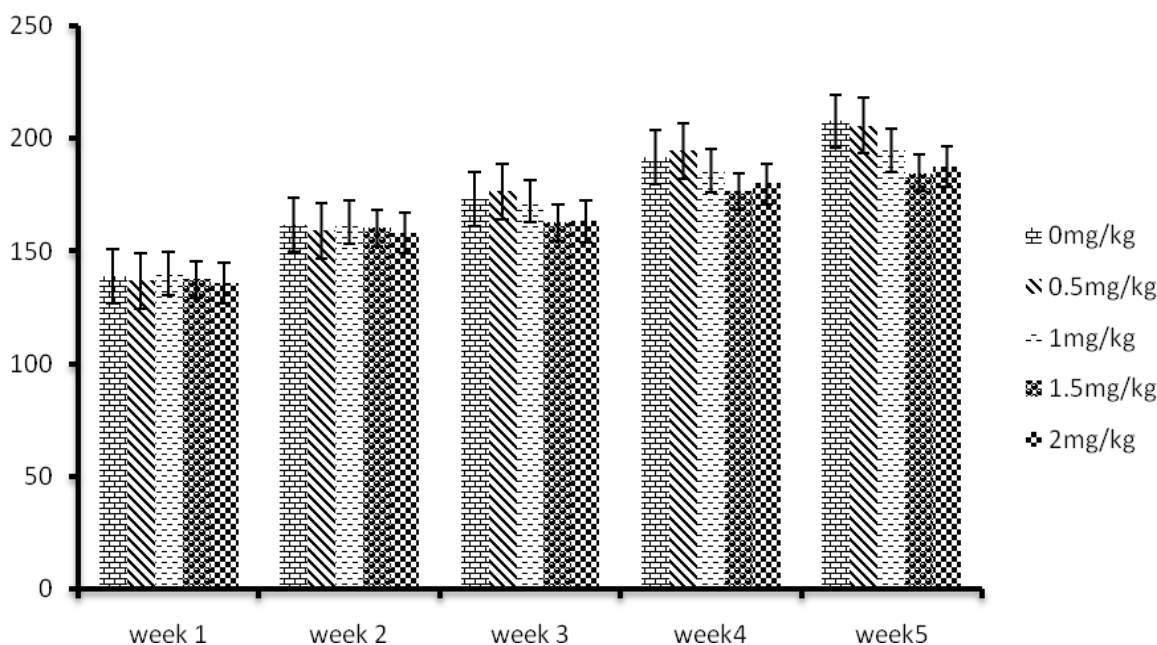


Figure 2: Average weight gain of wistar rats treated with varying doses of spinosad

CONCLUSION

Spinosad as a formulated form “Tracers®” under the present experimental conditions seems to be toxic to liver at a particular dosage by varied changes in cyst observed and in the levels of the heamatology, biochemical parameters and histopathology changes as a result of

different concentrations in the treated groups relative to the control group. The effect of spinosad in this study was acute as the test rats only showed some clinical signs of pesticide poisoning at 10-15days of treatment but they recovered before the 21 days of treatment and there was no mortality recorded. From the outcome of this study, the parameters can be used as bio indicators of exposure and effects in instances where spinosad and other bio-

pesticides are used as post-harvest insecticides. All treated animal's shows gradual changes as an effect to the treatment except for animals treated with 2mg/kg which shows a level of resistance to the toxicity of spinosad. Such results may highlight the necessity of evaluating the toxic hazards of spinosad at a higher (storage) dosage. The variation in result that is in contrast with the work of McCormack, (2011) and Mansour et al. (2007) may be due to the variation in species, resistance to pesticide by various rats or immunity to pesticide: hence area of further research should be on molecular level to ascertain reasons why toxicity of spinosad is less pronounced at 2mg/kg as compared with 1.5mg/kg.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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