Effects of Sub-Lethal Doses of Pyriproxyfen, Fenitrothion and Spinosad on Certain Biochemical Systems of Male Albino Rats

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ABSTRACT

Toxicological effects of sub-lethal doses ($1/10^{th}$ of LD_{50} and initial residues) of pyriproxyfen, fenitrothion and spinosad were studied in male albino rats. The ratio of certain organ to body weight, some blood components and enzyme activities(plasma cholinesterase and alkaline phosphatase) were determined. One tenth of the LD_{50} of the three insecticides showed a significant decrease in the weight of kidneys, and spleen of the tested animals with respect to the total weight, while there was no significant change in the weight of the liver with respect to the total weight. Regarding the initial deposits of the three insecticides, the results showed that there was no significant difference on the ratio of liver to total body weight for spinosad and fenitrothion, while the initial deposit of pyriproxyfen showed a significant decrease in the weights of liver, spleen and kidney with respect to the total body weight.

One tenth of the LD_{50} had insignificant effect for the insecticides fenitrothion and spinosad mostly on the number of red and white blood cell counts, while the dose of pyriproxyfen significantly reduced the number of white cells from 13 x 10^3 cell/ml in the control treatment to 4.3X10^3 cell/ml. The three insecticide treatments showed a significant increase in hemoglobin in comparison to the control. There was no significant effect for both doses of $1/10^{th}$ the LD_{50} and the initial deposit on Hematocrite concentration and the average volume of the red blood cells, except for the doses$1/10^{th}$ of the LD_{50} of spinosad and initial deposit of fenitrothion in which there was a significant increase in the average volume of the red cells in comparison to the control.

The two doses of insecticides showed a little inhibition of cholinesterase activity which ranged from 9.61 to 38.46% for both doses of the three insecticides. A significant increase in the activity of the enzyme alkaline phosphatase was recorded. Creatinine level was increased when animals were treated with both doses of pyriproxyfen. Fenitrothion treatment showed a decrease in creatinine level. The treatment of $1/10^{th}$ of LD_{50} of spinosad did not affect the level of creatinine, while the initial residue of spinosad showed a significant increase with respect to the control.

***Keywords: Hematological parameters, Male albino rats, Alkaline phosphatase, Cholinesterase*.**

INTRODUCTION

Pesticides are occasionally used indiscriminately in large amounts causing environmental pollution. Residual amounts of organophosphate (OP) and organochlorine (OC) pesticides have been detected in the soil, water, vegetables and grains and other food products (John *et al*., 2001). Toxicities of OP pesticides cause adverse effects on hematological and biochemical parameters (De Blaquiere *et al*., 2000). Fenitrothion is an OP insecticide used to control a variety of insects. It has been widely used throughout the world with applications in agriculture and horticulture for controlling insects in crops (Shioda *et al*., 1993). OP is known to cause inhibition of acetylcholinesterase (AChE) activity in the target tissues (Kappers *et al*., 2001). Pyriproxyfen is IGRs with a different mode of action, commonly used in crops to the control of lepidopteran pests. Spinosad is biopesticides isolated from a soil actinomycete and found to have a new neurotoxic mode of action (Yeh *et al.*, 1997) Toxicity of pesticides affects many organs, particularly, brain, liver and kidney. Bagchi *et al*., 1992 and El- Shahawi *et al.* 1999, reported an increase in liver and spleen related with body weight when rats and mice treated with endrin and acetamiprid. El-Gendy (1991), reported decrease in the body weight and spleen and an increase in the ratio of liver, kidney and brain related to body weight. Radwan *et al* (2001), reported that 1/10 of LD_{50} of pyriproxyfen, azdrachtin and fenitrothion increased the blood components of WBC and RBC. Also, Al-Rajhi *et al.* (1999), showed an increase of WBC & MCV and slight decrease of RBC & hemitocrite percentage, while hemoglobin concentration did not affected after diazinon and pirimiphos-methyl treatments. Plasma cholinesterase (ChE) was inhibited while Alkaline phosphatase (ALP) increased in the rat serum when rat treated with sub lethal doses of Organophosphates (Op) (EL-Elaimy *et al.,* 1988). Abdel-Megeed *et al.* (2001) mentioned that the $1/10^{th}$ of LD_{50} of fenitrothion decreased the ALP of rat while azdrachtin and pyriproxyfen increased the activity of the enzyme. Radwan *et al*. (2001) reported that male rat that treated orally with $1/10^{th}$ of LD_{50} of fenitrothion, cypermethrin and pyroproxyfen increased the level of creatinine content. Also, Abd El-Aziz (2000) and El-Aswad (2001) reported an increase of creatinine level in serum of rat that treated with Op and carbamates. The chlorpyrifos exposure caused excess of weight gain in males beginning at postnatal day (PND) 45 and reaching

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levels 10.5% above control by PND 72 (Lassiter and Brimijoin, 2008).

The aim of this work was to study the toxicological effects of sub lethal doses of pyriproxyfen, fenitrothion and spinosad on certain biochemical systems of male albino rats.

MATERIALS AND METHODS

Animals:

Male albino rats, *Rattus ratus norvigenous* (170-180 gm) were obtained from Faculty of Pharmacy, King Saud University. Animals were housed in stainless steel cages and provided with food and water *ad lib*. All animals were maintained on 12 h light/12 h dark cycle at constant temperature (22 ± 1.0 ^{o}C).

Chemicals:

The following insecticides were purchased locally and used. Admiral (pyriproxyfen, 10% EC, Sumitomo Co.); Sumithion (fenitrothion, 50% EC, Sumitomo Co.) and Tracer (spinosad, 48 % SC, Dow Agrosciences Co).

Experimental protocol:

Rats were divided randomly into 7 groups each of 9 rats. Group 1. control treated with tap water once a week. Group 2. treated with 1/10 LD_{50} of pyriproxyfen (500 mg /kg b.w) once a week for four weeks. Group 3. treated with initial residue (zero time) of pyriproxyfen (6.71 mg/kg b.w) daily for one month. Group 4. treated with 1/10 LD_{50} of fenitrothion (24 mg /kg b.w) once a week for four weeks. Group 5. treated with initial residue of fenitrothion (3.48 mg/kg b.w) daily for one month. Group 6. treated with 1/10 LD_{50} of spinosad (500 mg /kg b.w) once a week for four weeks. Group 7. treated with initial residue of spinosad (0.52 mg/kg b.w) daily for one month. All doses were provided orally using stomach tubes. All animals were weighed at the beginning and the end of experiment and the change of body weight were determined. Organ weight index of lymphatic organ (spleen), parenchymatous organs (liver and kidney) were weighed and calculated as weight indices as organ body weight ratio according to Bronisz *et al*., (1992).

Hematological studies:

Five rats from each group were randomly selected after one month from treatments and anesthetized with Et_2O and blood was withdrawn via retro-orbital plexus using a heparinized microcapillary tube (El-Shahawi, 1996). Blood samples were collected from each animal in 5 ml citrated tubes containing anticoagulants (120 mM trisodium citrate). Red blood cell (RBC), white blood cell (WBC), Hemoglobin (HGB) concentration, Hematocrite concentration (HCT) and mean cell volum (MCV) were measured using Hemacomp 5 instrument.

Enzyme assay:

Cholinesterase (CHE) activity in plasma was determined according to (Knedel and Bottler (1967) using butyryl thiocholine as substrate. Alkaline phosphatase (ALP) in plasma was determined according to REC. GSCC (DGKC), 1972, using p-nitrophenyl phosphate as substrate. Creatinine concentration was determined according to Schirmeister *et al*., (1964).

Analysis of data:

The data was subjected to statistical analysis (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Body organs and Hematological studies:

The toxicological effect of 1/10 th of LD_{50} and initial residues of pyriproxyfen, fenitrothion and spinosad on the ratio of certain organ to body weight of male albino rate was shown in Table (1). The dose of 1/10th of LD_{50} of pyriproxyfen (500mg/kgbw), fenitrothion (24mg/kgbw) and spinosad (500mg/kgbw) was found to decrease the percentage of weight gain of the tested animals significantly. The initial residue of spinosad (0.52 mg/kg) and pyriproxyfen (6.7 mg/kg) showed a significant decrease in the gain of weight percentage compared to the control, while the initial deposit of fenitrothion (3.48 mg/kg) show a significant increase on weight gain percentage. One tenth of the LD_{50} of the three insecticides showed significant decrease in the weight of kidneys and spleen of the tested animals with respect to the total weight, while there was no significant change in the weight of the liver with respect to the total weight. Regarding the initial deposits of the three insecticides, the results showed that there was no significant difference on the ratio of liver to total body weight for spinosad and fenitrothion treatments, while the initial deposit of pyriproxyfen showed a significant decrease in the weights of the three organs with respect to the total body weight. The results in agreement with that reported by (El-Gendy, 1991 ; Bronisz *et al*, 1992 , Al-Rajhi *et al*., 1999 and El-Shahawi *et al*., 1999) they found a decrease in spleen and liver when treated with sub lethal doses of pesticides. In contrast, Neskovic *et al*., 1989 pointed to an increase of spleen when rat treated with pirimiphos-methyl. Moreover, El-Aswad (2001), reported non significant increase of spleen when rats treated daily for 90 days with sub lethal doses of pirimiphos-methyl and profenfos. The chlorpyrifos exposure caused excess weight gain in males beginning at postnatal day (PND) 45 and reaching levels 10.5% above control by PND 72 (Lassiter and Brimijoin, 2008).

Table 1. Effect of 1/10 of LD_{50} and initial residues of pyriproxyfen, fenitrothion and spinosad on the ratio of certain organ to body weight of male albino rate

Treatments	Body weight	Liver weight	W*	Kidney Weight	W	Spleen weight	W
Control	282.5 ± 11.0	14.49 a ± 1.7	5.13	1.20 a ± 0.1	0.42	1.93 a ±0.3	0.68
Pyriproxyfen 1/10 LD_{50} (500mg/kgbw)	268.2 ± 9.2	13.05ab ± 0.2	4.87	1.04bc ± 0.1	0.39	0.71 c	0.26
Initial residue (6.7 mg/kg)	271.4 ± 12	11.72c ± 1.1	4.32	0.93 c ± 0.1	0.34	1.05 b ± 0.1	0.39
Fenitrothion 1/10 LD_{50} (24mg/kgbw)	266.6 ± 8.7	12.11 bc ± 3.2	4.54	1.04 bc ± 0.1	0.39	1.16 b ± 0.2	0.44
Initial residue (3.48 mg/kg)	308.2 ± 10.8	14.42 ab ± 1.5	4.61	1.09 ab ± 0.1	0.35	1.03 b ± 0.2	0.33
Spinosad 1/10 LD_{50} (500mg/kgbw)	266.0 ± 8.0	12.76 abc ± 0.9	4.8	1.05 bc± 0.1	0.39	1.18 b± 0.1	0.44
Initial residue (0.52 mg/kg)	279.4 ± 7.8	12.30 abc ± 0.7	4.40	0.97 c ± 0.1	0.35	1.07b ± 0.2	0.38
$LSD_{0.05}$		2.26		0.122		0.229	

*W = {Organ weight (gm)/ Body weight (gm)} X 100.
* same liters mean no significant difference.

Toxicological effect of $1/10^{th}$ of LD_{50} and initial residues of pyriproxyfen, fenitrothion and spinosad on certain blood components of male albino rats was illustrated in Table (2). Red blood cell (RBC), white blood cell (WBC), Hemoglobin (HGB) concentration, Hematocrite concentration (HCT) and mean cell volume of red blood cell (MCV)was measured using Hemacomp 5 instrument. $1/10^{th}$ of the LD_{50} had insignificant effect for the insecticides fenitrothion and spinosad mostly on the number of RBC and WBC counts, while the dose of pyriproxyfen significantly reduced the number of WBC from 13 x 10^3 cell/ml in the control treatment to 4.3X10^3 cell/ml. The three insecticide treatments showed significant increase in HGB in comparison to the control. There was no significant effect for both doses; $1/10^{th}$ of LD_{50} and the initial deposit on the percentage of HCT. The average volume of the red blood cells did not affect significantly by both doses, except for the doses$1/10^{th}$ of the LD_{50} of spinosad and initial residues of fenitrothion in which there was a significant increase in the MCV in comparison to the control. The results in line with that reported by (Enan, 1976, Gupta *et al*., (1982), El-Bakry, 1994 and Radwan *et al*., (2001) they reported an increase in number of WBC in rat blood that treated with sub lethal doses of insecticides. They referred that the increase of WBC as a results of the disease effect of insecticides. In contrast, El- Khatib (1986); Rajini *et al.*, 1987 and Al-Rajhi *et al.*, 1999, reported decrease in RBC in rats that treated with sub lethal doses of Althrin, pirimiphos-methyl and cypermethrin.

The side effect of the two doses of insecticides on some enzyme activity was also studied (Table 3). There was little inhibition of the activity of the enzyme cholinesterase (ChE) in rat plasma which ranged from 9.61 to 38.46% for both doses of the three insecticides: There was significant increase in the activity of the enzyme alkaline phosphatase (ALP) in rat plasma which reached more than three folds of the control treatment for $1/10^{th}$ of the LD_{50} dose of spinosad. The concentration of creatinine was increased with respect to the control treatment when animals were treated with both doses of pyriproxyfen. The treatment of fenitrothion showed decrease in the creatinine concentration. The treatment of $1/10^{th}$ of LD_{50} of spinosad did not affect the concentration of creatinine, while the initial residue of spinosad showed significant increase in creatinine concentration with respect to the control. The results in agreement with that reported by Davis and Holub (1980) and El-Bakary (1994) in which the plasma ChE activity in rat decreased as a results of sub lethal treatment with insecticides. El-Elaimy *et al.*, (1988); Abd-El-Aziz (2000) and Abdel-Megeed *et al*., (2001) reported an increase in ALP activity after insecticide treatments. In the case of creatinine concentration the obtained results of the three insecticides in the normal range. Schirmeister *et al.*, (1964) point that a range of 53- 97 umole /L has no negative effect on human health. Yehia *et al*., 2007, reported that, exposed of rabbits to diazinon caused extensive changes in physiological, biochemical, and histopathological parameters as well as

Table 2. Effect of 1/10 of LD_{50} and initial residues of pyriproxyfen, fenitrothion and spinosad on certain blood components of male albino rats

Treatments	RBC 10^6 cell/ml	WBC 10^3 cell/ml	HGB gm/100ml blood	HCT (%)	MCV Micron/RBC
Control	8.0 ab ± 1.0	13.0 a ± 3.4	17.7b ± 5.3	75.4ab ± 7.5	81.8 c ± 2.1
Pyriproxyfen 1/10 LD_{50} (500mg/kgbw)	7.1 b ± 1.4	4.3 b ± 1.4	23.8 a ± 2.9	58.5 b ± 12.3	82.8 bc± 0.3
Initial residue (6.7 mg/kg)	7.5 ab ± 0.3	12.5 a ± 3.8	25.0 a ± 0.6	64.5 ab ± 1.8	86.0 abc ± 2.8
Fenitrothion 1/10 LD_{50} (24mg/kgbw)	7.5 ab ± 1.0	15.6 a ± 3.8	22.4 a ± 6.0	63.5 b ± 7.3	85.1 abc ± 0.2
Initial residue (3.48 mg/kg)	8.4 a ± 0.6	15.7 a ± 3.3	26.0 a ± 6.0	73.9 a ± 5.5	89.6 a ± 5.0
Spinosad 1/10 LD_{50} (500mg/kgbw)	7.7 ab ± 0.5	14.1 a ± 2.4	24.6 a ± 0.5	67.3 ab± 3.4	87.2 ab ± 4.2
Initial residue (0.52 mg/kg)	7.7 ab± 1.2	14.0 a ± 3.6	25.7 a ± 1.8	65.6 ab ± 8.8	85.7 abc ± 4.7
$LSD_{0.05}$	1.18	4.15	4.28	9.58	4.63

Red blood cell (RBC), white blood cell (WBC), Hemoglobin (HGB) concentration, Hematocrite concentration (HCT)and mean cell volum (MCV).
*same liters mean no significant difference.

Table 3. Effect of 1/10 of LD_{50} and initial residues of pyriproxyfen, fenitrothion and spinosad on creatinine concentration, Choline esterase and alkaline phosphatase activities in blood plasma of male albino rats

Treatments	*ChE activity (unit/L)	% Activity of control	**ALP	% Activity of control	***Creatinine concentration	% change of control
Control	1524.9 ± 165.9	100	60.1 ± 21.3	100	66.95 ± 13.6	100
Pyriproxyfen 1/10 LD_{50} (500mg/kgbw)	1231.7 ± 126.7	80.73	88.0 ± 28.0	146.4	102.55± 15.2	153.1
Initial residue (6.7 mg/kg)	1192.6 ± 27.6	78.21	94.9 ± 13.7	157.9	100.71 ± 6.1	150.4
Fenitrothion 1/10 LD_{50} (24mg/kgbw)	938.4 ± 37.1	61.54	156.8 ± 68.5	260.9	57.93 ± 6.1	86.5
Initial residue (3.48 mg/kg)	938.4 ± 71.8	61.54	138.6 ± 22.4	230.6	61.11 ± 7.9	91.3
Spinosad 1/10 LD_{50} (500mg/kgbw)	1378.3 ± 50.8	90.39	189.8 ± 37.8	315.8	66.75 ± 10.2	99.7
Initial residue (0.52 mg/kg)	1202.3± 97.3	78.84	156.8 ± 37.8	260.9	73.94 ± 6.5	110.44

*Normal value of Ch E activity at 25 ^oC (3500-8500 Unit/L)
**Normal value of ALP activity at 25 ^oC (60-170 Unit/L)
***Normal concentration of creatinine 25 ^oC (53-97 uMole/L)

histochemical AChE. So, contact exposure of diazinon leads to negative response on animal health.

It could be concluded that the three insecticide treatments at the applied doses had no severe effect on kidney, liver and/or the enzyme activities and do not pose threat to human health since there activities are still within the normal ranges. Also, the insecticide treatments do not pose a health threat to humans regarding the concentration of creatinine since the concentration of creatinine was still within the normal range, except the $1/10^{th}$ dose of LD_{50} of pyriproxyfen (102.52 umole/L) and the initial deposit of the same insecticide (100.71 umole/L).

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الملخص العربي

تأثير جرعات تحت مميتة من البيريبروكسفين والفينتروثيون والسبينوساد على بعض النظم البيوكيميائية لذكور فئران الألبينو

سليمان بن عبد الكريم بن على المحيميد، ضيف الله بن هادي الراجحى، علاء صلاح الدين كامل

تم دراسة التأثيرات التوكسيكولوجية للجرعات تحت المميتة (عشر الجرعة المميتة ل 50% من الفئران و المتبقيات الأولية على الخضروات) من المبيدات الحشرية البيريبروكسفين والفينتروثيون والسبيونوساد على ذكور الفئران من النوع الألبينو. تم تقدير نسبة التغير في بعض الأعضاء إلى وزن الجسم وبعض مكونات الدم ونشاط إنزيميّ الكولين استيريز في البلازما والفوسفاتيز القاعدي. أظهر عشر الجرعة القاتلة ل50% من المبيدات الثلاث انخفاض في وزن كل من الكلية والطحال في الحيوانات المعاملة بينما لم تظهر تغيرات معنوية في وزن الكبد منسوباً إلى وزن الجسم. لم تسجل أي تغيرات معنوية لتأثير المتبقيات الأولية لمبيديّ السبينوساد والفينتروثيون على نسبة الكبد إلى وزن الجسم الكلي. بينما المتبقيات الأولية لمبيد البيريبروكسفين أظهرت زيادة معنوية في وزن كل من الكبد والطحال والكلية.

أظهر عشر الجرعة القاتلة ل50% من مبيد الفينتروثيون تأثيرات غير معنوية على أعداد كل من كرات الدم الحمراء والبيضاء بينما جرعة مبيد البيريبروكسفين خفضت معنوياً أعداد كرات الدم البيضاء من13×10^{3} في المقارنة إلى 4.3×10^{3}خلية/مل بالمعاملة. لم يظهر تأثيرات معنوية للجرعات المستخدمة على أي من مستوى الهيماتوكريت ومتوسط حجم كرات الدم الحمراء ما عدا عشر الجرعة القاتلة ل50% من مبيديّ السبينوساد والمتبقيات الأولية للفينتروثيون حيث أزداد حجم كرات الدم الحمراء معنوياً مقارنة بالغير معاملة.

وأظهر الجرعتين من المبيدات تأثيرات تثبيطية طفيفة على نشاط الكولين استيريز بالبلازما حيث تراوحت بين9.61% إلى38.46 % للمبيدات الثلاث. بينما أظهرت زيادة في نشاط إنزيم الفوسفاتيز القاعدي. وأزداد مستوى الكرياتينين في الفئران المعاملة بجرعتي البيريبروكسفين. بينما أظهرت المعاملة بمبيد الفينتروثيون انخفاض في مستوي الكرياتينين. لم يؤثر عشر الجرعة القاتلة ل50% من مبيد السبينوساد على مستوي الكرياتينين. بينما أظهرت المتبقيات الأولية من السبينوساد زيادة معنوية في مستوى الكرياتينين مقارنة بالغير معاملة.