Acute Toxicity Impacts of Diazinon and Lambda Cyhalothrin on Mal Albino Rat

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ABSTRACT

The present study was carried out to investigate the impact of single sublethal oral dose of 36.5 mg/kg bw DZ(1/10 LD_{50}; 365 mg/ kg bw) and 2.34 mg/kg bw LC(1/10 LD_{50}; 23.4 mg/ kg bw) individually for 24 hr on some biochemical, hematological and immunological parameters in rat serum. Results showed that DZ increase aspartate aminotransferase(AST) and alanine aminotransferase(ALT) activities, uric acid and creatinine concentration, while it reduced total cholesterol level. LC treatment increased serum; AST and ALT activities, uric acid, creatinine and total cholesterol levels, while the triglycerides levels were significantly reduced. Also, Hematological studies revealed that DZ treatment significantly increase WBCs and RBCs counts and Hb content, while Hct and PLT values showed no significant changes compared to control. However, LC treatment didn't induce any significant changes in all peripheral blood cell indicators except the Hct value which exhibited a significant elevation compared to control. Both DZ and LC treatment showed no significant alterations in the erythrocytes indices. Immunological studies showed that DZ caused significant decrease in lymphocytes counts($p < 0.05$), while the peripheral neutrophils percentage and monocytes counts were significantly increased. LC treatment enhanced the peripheral neutrophils and monocytes percentage, while lymphocytes counts showed no significant alteration. The present result concluded that these insecticides have adversely affect biomarkers of rat serum, so it must be used wisely and carefully in order to prevent the hazardous side effects on mammalian targets.

Keywords: Diazinon, lambda cyhalothrin, Serum, Hematological studies, Immunological parameters.

INTRODUCTION

Pesticides are widely used in order to enhance the food protection by controlling the unwanted insects and disease vectors. Diazinon(DZ), is an organophosphorous insecticide which is widely and effectively used throughout the world with applications in agriculture and horticulture for controlling insect pests in crops, ornamentals, lawns, fruit, vegetables and other food products (Tang et al, 2009; Sarabia et al, 2009). DZ can be highly toxic for animals and human being even after a single exposure (Poet et al, 2004; Sarabia et al, 2009). Several investigations have showed that DZ was capable of inducing biochemical and hematological alterations (Al-Attar, 2009, Al-Attar and Al-Taisan, 2010; Al-Attar and Abu Zeid, 2013). Significant damage in the hepatic cells and glucose metabolism in liver was observed as the result of diazinon administration (Fatima et al, 2006). The toxicological effects of DZ in animals and human have been demonstrated in acute and chronic exposures, changes in liver enzymes and biochemical indices and swelling of mitochondria in hepatocytes (Kalender et al, 2005).

Lambda cyhalothrin (LC) is a potent synthetic type II pyrethroid used worldwide to control a wide range of insect pests in agriculture, forestry, public health and disease vector control (Fetoui et al, 2009 and Rachid et al, 2010). Residues of LC have been reported in vegetables and fruits(Turgut et al, 2011), milk and blood of dairy cows(Bissacot and Vassilieff, 1997) and also in cattle meat(Muhammad et al, 2010). Blood findings are important for the assessment of various systemic functions and health of animals under various environmental conditions and most importantly for diagnosis of drug or chemical induced haemolysis (Atamanalap and Yanik, 2003). The present study aimed to evaluate the adverse effect of a single sublethal oral dose of diazinon and lambda cyhalothrin on some biochemical, hematological and immunological parameters in rat serum, consequently these parameters can be used as potential biomarkers of liver and kidney damage caused by diazinon and lambda cyhalothrin.

MATERIALS AND METHODS

Chemicals

Diazinon (Diazomax 60 % EC) O,O-Diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate was obtained from Egyptchem International for Agrochemicals Nubarya city, Behera, Egypt, Lambda cyhalothrin (Katron 5% EC) [*R*)-α-cyano-3-phenoxybenzyl (1*S*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethyl cyclopropane carboxylate and (*S*)-α-cyano-3-phenoxybenzyl (1*R*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2 dimethyl cyclopropane carboxylate] was obtained from National Agricultural Chemicals and Investment (El-Watania Co) Nubarya, Behera, Egypt. All other chemicals used were of analytical grade purchased from Sigma – Aldrich Co. Saint Louis, Missouri, USA.

Animals

Healthy male albino rats weighing 150 -160 g were obtained from the animal house, Faculty of Agriculture, Alexandria University. Animals were housed in groups each of five rats in stainless steel cages and kept under laboratory conditions (25±°C, R.H 65 –75% and light dark cycle, 12 h light, 12 dark). Animals were given feed and water ad libitum 24 hr / day. The animals were acclimatized to laboratory conditions for two weeks before being experimented. All procedures involving animals were performed in accordance with Organization for Economic Cooperation and Development, Acute Oral Toxicity Study in Rodents: OECD guideline, 420 (2001).

Experimental design

Animals were divided into three groups each of five rats and orally treated with single dose which represent 1/10 LD_{50} of either DZ or LC via stomach gavage tube for as follow:

Group I: Rats were administered with water and served as control.

Group II: Rats were administered with 36.5 mg/kg bw DZ (1/10 LD_{50}; 365 mg/ kg bw) in water.

Group III: Rats were administered with 2.34 mg/kg bw LC (1/10 LD_{50}; 23.4 mg/ kg bw) in water. The oral LD_{50} for DZ and LC were estimated according to the protocol of Acute Oral Toxicity Study in Rodents: OECD guideline, 420(2001) by the Department of Mammalian Toxicology, Pesticide Central Laboratory, Agriculture Research Center, Egypt. All animals were observed for 14 days after the treatment.

Blood sample collection

Blood samples were collected from orbital vein of rats and placed immediately into two tubes; the first tube containing ethylenediaminetetra acetic acid(EDTA) as anticoagulant for hematological analysis and the second tube without anticoagulant for serum preparation. Non-coagulated blood samples were used for peripheral blood cell indicators determination such as white blood cells(WBCs) and red blood cells(RBCs) count, haemoglobin content(Hb), Hematocrite percentage(Hct) and total thromocyte(platelet)(PLT) count as described by Dacie and Lewis(1991). Erythrocyte indices including mean corpuscular volume(MCV), mean corpuscular haemoglobin(MCH) and mean corpuscular haemoglobin concentration(MCHC) were calculated. Also, the leukocyte formula; the peripheral neutrophils percentage, lymphocytes and monocytes counts were determined. For serum preparation blood samples were allowed to stand for 30 min at room temperature till clotted and centrifuged at 4,000 x g using Sigma 3K30 Bench Centrifuges for 15 min to separate the serum; then kept at -20^{o}C for the biochemical studies.

Biochemical studies

Sera samples were used to estimate the activities of aspartate and alanine aminotransferase(AST& ALT) according to the method of Retiman and Frankel(1957), whereas total bilirubin, urea and creatinine concentrations were determined according to the methods of Walter and Gerade (1970); Fawcett and Scott (1960) and Schirmeister *et al*,(1964) respectively. Cholesterol and triglycerides levels were determined by the methods of Richmond, 1973 and Fassati and Prencipe (1982) respectively, Standard Biodiagnostic kits were purchased from Bio-diagnostic, 29 El Tahrir St., Dokki, Giza, Egypt .

Statistical analysis

All data were expressed as mean ± standard deviation(SD). Data were analyzed using one-way analysis of variance(ANOVA) followed by the Student–Newman–Keuls test to determine significance between different groups using a computer software CoStat program. The criterion for statistical significance was set at $p<0.05$.

RESULTS

Symptoms of toxication

No mortality occurred in any group throughout the experiment. Some cases of DZ treated rats showed lameness, weakness and diarrhea, while LC treated rats showed some signs of toxicity included; piloerection, subdued behavior, ataxia, unsteady gait, salivation and incontinence.

Biochemical studies

The acute toxicity effects of single sublethal dose of DZ and LC on some biochemical, hematological and immunological parameters in rat serum were investigated as follow.

Serum biomarkers:

AST and ALT activities*:*Data in Table (1) show a significant elevation ($p<0.05$) in the activities of AST and ALT by 26% and 21%, respectively in rat treated with DZ, whereas rat treated with LC exhibited significant elevation by 27.6% in the AST activity and reduction in ALT activity by 13% compared to control.

Total bilrubin levels*:* Table (1) revealed significant elevation ($p<0.05$) in the total bilrubin level by 62.5% and 81% in rat treated with DZ and LC respectively compare to control.

Changes in cholesterol and triglycerides levels:

Administrations of DZ and LC caused significant alterations in the serum lipids; the total cholesterol concentration exhibited significant reduction ($p<0.05$) by 85% in rat treated with DZ while, rat treated with LC showed a significant elevation by 37.5%. The triglycerides level showed non significant alteration in rat treated with DZ, at meantime rat treated with LC showed a significant reduction by 43.4% compared to control (Table 1).

Creatinine and uric acid levels: As revealed in Table (1) creatinine and uric acid levels were significantly increased in all tested insecticides compared to control levels.

Haematological studies

As shown in Table (2) DZ treatment induced significant increase in WBC's and RBC's counts and Hb content, while Hct and PLT values showed non significant changes compared to control. The LC treatment didn't affect significantly the peripheral blood cell indicators except the Hct value which exhibited a significant elevation compared to control. Data in Table (3) revealed that both DZ and LC had no significant effects on the erythrocytes indices.

Immunological parameters

The experimental data on leukocyte formula showed that treatment with DZ induced a marked decrease in the lymphocytes counts (Table 4), whereas the peripheral neutrophils percentage and monocytes counts were significantly increased ($P<0.05$). LC treated group exhibited an enhancement in the peripheral neutrophils and monocytes percentage while lymphocytes counts showed no significant alteration compare to control.

Table 1: Serum biomarkers of male rats orally administrated to single sub-lethal dose of 36.5 mg/ kg bw DZ and 2.34 mg / kg bw LC.

Animal Group	Parameters						
	AST (U/ml)	ALT (U/ml)	Bilrubin (mg/dl)	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control (I)	$210^{a} \pm 4.5$	$260^{b} \pm 8.2$	$0.80^{a} \pm 0.03$	$152^{b} \pm 2.1$	$136^{b} \pm 4.2$	$17.5^{a} \pm 0.25$	$3.82^{a} \pm 0.24$
DZ (II)	$265^{b} \pm 4.8$	$315^{c} \pm 9.5$	$1.31^{b} \pm 0.01$	$23^{a} \pm 3.4$	$137^{b} \pm 4.2$	$21.4^{b} \pm 0.21$	$10.88^{b} \pm 0.48$
LC (III)	$268^{b} \pm 8.2$	$226^{a} \pm 4.5$	$1.45^{b} \pm 0.05$	$209^{c} \pm 2.2$	$77^{a} \pm 4.1$	$20.8^{b} \pm 0.21$	$9.56^{b} \pm 0.66$

Values are expressed as means (5 rats) ± standard deviation (SD)
Values in column with different letters are significantly different at ($p \leq 0.05$).

Table 2: Peripheral blood cell indicators of male rats orally administrated to single sub-lethal dose of 36.5 mg / kg bw DZ and 2.34 mg / kg bw LC.

Animal Group	Parameters				
	WBC (10^3 / µl)	RBC (10^6 / µl)	Hb (g/dl)	Hct (%)	PLT (10^3/ µl)
Control (I)	$17.3^{b} \pm 1.11$	$4.34^{a} \pm 0.31$	$11.16^{a} \pm 1.03$	$36.02^{a} \pm 0.91$	$979.0^{a} \pm 55.34$
DZ (II)	$24.48^{c} \pm 1.23$	$5.26^{b} \pm 0.24$	$13.34^{b} \pm 0.21$	$41.54^{a} \pm 1.62$	$1051.8^{a} \pm 39.23$
LC (III)	$12.43^{a} \pm 0.60$	$4.56^{a} \pm 0.21$	$12.56^{a} \pm 0.56$	$42.43^{b} \pm 2.00$	$718.3^{a} \pm 14.60$

Values are expressed as means (5 rats) ± standard deviation (SD)
Values in column with different letters are significantly different at ($p \leq 0.05$)

Table 3: Erythrocytes indices of male rats orally administrated to single sub-lethal dose of 36.5 mg / kg bw DZ and 2.34 mg / kg bw LC.

Animal Group	Parameters		
	MCV (fL)	MCH (Pg)	MCHC (g / dL)
Control (I)	$84.12^{a} \pm 6.64$	$25.2^{a} \pm 2.42$	$31.00^{a} \pm 0.93$
DZ (II)	$77.26^{a} \pm 6.75$	$24.84^{a} \pm 2.43$	$31.98^{a} \pm 1.02$
LC (III)	$91.00^{a} \pm 3.60$	$26.40^{a} \pm 1.30$	$30.00^{a} \pm 1.00$

Values are expressed as means (5 rats) ± standard deviation (SD)
Values in column with different letters are significantly different at ($p \leq 0.05$).
MCV = Hct / RBC. Reporting units is femtoliters (fL).
MCH = Hb / RBC. Reporting units is picograms (pg).
MCHC = Hb / Hct. Reporting units is grams / deciliter (g / dl).

Table 4: Immunotoxic potential of single sub-lethal dose of 36.5 mg / kg bw DZ and 2.34 mg / kg bw LC: The Leukocyte Formula.

Animal Group	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
Control (I)	76 ± 1.58^{b}	8.4 ± 0.55^{a}	14.6 ± 1.14^{a}
DZ (II)	70.2 ± 1.48^{a}	10 ± 0.71^{b}	17.6 ± 1.14^{b}
LC (III)	76.8 ± 0.83^{b}	9.48 ± 0.083^{b}	19.36 ± 0.58^{c}

Values are expressed as means (5 rats) ± standard deviation (SD)
Values in column with different letters are significantly different at ($p \leq 0.05$).

DISCUSSION

The results showed that there was no mortality due to either DZ or LC exposure. However, some cases of DZ treated rats showed lameness which may be due to organophosphorous pesticides poisoning which are known to induce toxicity in mammals by inhibiting acetyl cholinesterase resulting in accumulation of acetylcholine at the receptors(Hazarika et al, 2003). Similar results were obtained by Veerramachaneni et al,(2006). In case of LC rats showed some signs of toxicity included piloerection, subdued behavior, ataxia, unsteady gait, salivation and incontinence, characteristics of type II pyrethroid toxicity.

Liver plays an important role in the detoxification processes and along with kidneys faces the threat of maximum exposure to xenobiotics and their metabolic byproducts. Measurement of blood biochemical parameters is used as important diagnostic tool for the detection of abnormalities in the liver and other tissues(Banaee et al, 2011). Enzymes such as AST and ALT were considered to be sensitive indicators of hepatocellular damage (Peng et al, 2007).. The present results revealed that DZ and LC treatment caused a significant increase in the activities of AST and ALT. The elevation in the liver enzyme activities may be due to liver dysfunction with a consequent reduction in enzyme biosynthesis and altered membrane permeability permitting enzyme leakages into the blood (Mansour and Mossa 2010). As certain hepatic damage is considered pathologically irreversible (Helling et al, 1995), elevated level of ALT in the serum is a prominent index of liver damage and may render the liver to be more susceptible to other pathogen/toxicants(Nayak et al, 1996). In the present study, the increase in total bilirubin level in DZ and LC exposed rats may be due to increased haemoglobin percentage resulted from increased destruction of red blood cells. The pesticides may also block the biliary tract in treated rat which is another possible mechanism of their toxicity. (Saxena and Sharma(1999) reported that in LC exposed rats, the rise in total serum cholesterol level could be due to obstruction in liver bile ducts causing decline or interruption of its secretion to the duodenum consequently producing cholestasis. The disturbance in lipoprotein formation is one of the factors leading to accumulation of cholesterol in pesticide treated rats(Hassan, 1995). In contrary, DZ toxication reduce serum cholesterol level which could be related to the changes in carbohydrate metabolism which is influenced by the adrenals' changes induced by DZ(Matin et al, 1989), who observed that single dose of diazinon caused hyperglycemia and induction of liver gluconeogenesis enzymes. Triglycerides are free fatty acids esters of glycerol. Liver is responsible for the biosynthesis and assimilation of lipoproteins like LDL and VLDL, where triglycerides are secreted into circulation(Shen, 1980). The rise of these lipoproteins increases serum triglyceride. The present investigation revealed no significant alteration in the triglyceride level in DZ treated group, whereas LC treatment reduced the triglyceride level.

The elevation in blood uric acid and creatinine is considered a specific and sensitive indicator of impaired kidney function caused by oxidative stress. In this study, DZ and LC treatments induced a significant rising in the uric acid and creatinine concentration which in agreement with(Ahlam, 2009) by dosing rats with organophosphrous pesticide(profenofos) 1/20 LD_{50}. This increase may be relate to either increase in protein degradation, that's involved in uric acid formation, or to the toxic effect of pesticides on the kidney (Eraslan et al, 2007). Janardhan et al,(1988) observed an increase in blood uric correlated with histopathological degenerative changes in the kidney and these changes caused disturbance in the transport system of biochemical constituents. Low clearance values for creatinine and uric acid indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine(Cameron, 1996).

Blood is a sensitive index of the physiological changes of an animal to any environmental pollutants and it is well known that toxic stress of any nature would show conspicuous and significant changes in the hematological parameters. WBCs are important cells in the immune system, because of their main defensive function. The WBCs responses immediately to the change in medium due to toxicant. The present result showed that DZ treatment significantly increase WBCs counts and this may be attributed to the pesticide caused enteritis(Feldman et al, 2000). The significant reduction in the lymphocyts production was observed in DZ treatment may be due to the toxic effect of organophosphrous pesticides causing immunosuppressive effect (Ahlam, 2009).

CONCLUSION

The present study revealed that acute DZ and LC exposure resulted in biochemical and haematological changes in male albino rats. Therefore the present study calls for careful application of diazinon and lambda cyhalothrin in order to prevent the hazardous effects on humans and domestic animals.

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الملخص العربي

آثار السمية الحادة للديازينون ولامدا سيهالوثرين في ذكور الجرذان البيضاء

نادية على حامد، رضا خميس عبد الرازق

قسم بحوث سمية المبيدات للثديات – المعمل المركزى للمبيدات – مركز البحوث الزراعية – الاسكندرية

تم اجراء هذه الدراسة لبحث تأثير جرعة واحدة دون المميتة لكل من الديازينون بجرعة ٣٦.٥ ملجم / كجم من وزن الجسم (تمثل ١٠/١ LD_{50}، ٣٦٥ ملجم/ كجم من وزن الجسم) ولامدا بجرعة ٢.٣٤ ملجم / كجم من وزن الجسم (تمثل ١٠/١ LD_{50}، ٢٣.٤ ملجم / كجم من وزن الجسم) على بعض المؤشرات الحيوية فى دم ذكور الجرذان البيضاء. اوضحت النتائج ان التسمم بالديازينون أدى لزيادة معنوية فى نشاط انزيمى الأسبارتات واللأنين امينوترانسفيراز وكذلك زيادة تركيز كلا من حمض اليوريك، الكرياتينين وخفض مستوى الكوليسترول الكلي، في حين أن مستوى الدهون الثلاثية لم يتأثر معنويا. التسمم بمبيد لامدا سيهالوثرين أدى لزيادة معنوية فى نشاط انزيمى الأسبارتات والألنين امينوترانسفيراز وكذلك زيادة تركيز كلا من حمض اليوريك، الكرياتينين ومستوى الكولسترول الكلي وبشكل ملحوظ خفض مستوى الدهون الثلاثية. كما اوضحت الدراسة أن المعاملة بالديازينون أدت لزيادة معنوية فى كل من عدد كرات الدم البيضاء و الحمراء ومستوى الهيموجلوبين ولم تظهر اى تغيرات معنوية فى مستوى كلا من الصفائح الدموية و الهيماتوكريت مقارنة بالمجموعة الضابطة، في حين لم تظهر المعاملة بمبيد لامدا سيهالوثرين أي تغيير معنوى فى قيم مؤشرات خلايا الدم الطرفية، ماعدا زيادة معنوية فى مستوى الهيماتوكريت مقارنة بالمجموعة الضابطة. من ناحية اخرى فإن المعاملة بالديازينون او لامدا سيهالوثرين لم تحفز أي تغييرات معنوية فى مؤشرات كريات الدم الحمراء. كما أظهرت المؤشرات المناعية ان التسمم بالديازينون قد خفض معنويا عدد الخلايا الليمفاوية، بينما زاد معنويا عدد كلا من المونوسيت والنسبة المئوية للنتروفيل. وبالمثل التسمم بالامدا سيهالوثرين أدى لزيادة عدد كلا من المونوسيت والنسبة المئوية للنتروفيل بينما لم تظهر اى تغيرات معنوية فى عدد الخلايا الليمفاوية. وخلصت النتائج الى أن كلا من الديازينون ولامبدا سيهالوثرين قد تؤثر سلبا على المؤشرات الحيوية لدم الفئران ويمكن أن تضر الجهاز المناعى، لذا يجب أن يستخدما بحكمة وبعناية لمنع الآثار الجانبية الخطرة على الإنسان وحيوانات المزرعة.