Determination of blood cholinesterase level by electrometric method and inhibition by diazionon, dichlorvous and cypermethrin in vivo in homer pigeon (racing pigeon)

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Abstract

An environmental pollution with commonly used insecticides effected the homer pigeon this study used dichlirvous, diazinon and cypermethrin in several subacute doses The enzyme activity was measured by modified electometric method as ΔpH/ 30 min. The Confidence interval in the plasma were 0.197 respectively and those of the erythrocytes were 0.934 respectively. The organophosphorus insecticides diazinon at dose (5-10) mg\ kg b.w. significantly inhibited plasma (28~53%) and erythrocyte 53% and dichlorvous at dose (5-10) mg\kg b.w. significantly inhibited plasma (33~38%) and erythrocyte 52% cholinesterase *in vitro* in a concentration-dependent tanner. The insecticides cypermethrin at dose (20-40) mg\ kg b.w. significantly inhibited plasma lactic dehydrogenase in (4~9.5%).

تحديد فعالية انزيم الكولين استراز بالدم بالطريقة الكهرومترية بواسطة التثبيط بالديازينون والدايكلورفس والسايبر مثرين في داخل الجسم بطائر الزاجل

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الخلاصة

ان التلوث البيئي المصاحب لاستعمال المبيدات الحشرية الشائعة الاستعمال لها تأثير ضـــار لطـــائر الزاجـــل اشتملت هذه الدراسة على عدة مبيدات بالديازينون الدايكلورفس والسايبر مثرين بجرعات تحت الحادة متعـــدد وتـــم قياس مستوى انزيم الكولين استراز بواسطة الطريقة الكهرومترية المحورة من خلال تغير الباء هاء في 37 درجـــة مئوية حيث كان معامل الاختلاف في البلازما 0.179 وفي كريات الـــدم الحمـــراء 0.934، ان المبيـــد الحشـــري الديازينون ثبط الانزيم معنويا بنسبة (33–38%) في البلازما وبنسبة 52% في كريات الدم الحمراء بجرعـــة (5–10) ملغم/ كغم من وزن الجسم، أما المبيد الدايكلورفس ثبط الانزيم معنويا بنسبة (28–39%) في البلازما وبنســـبة 53% في كريات الدم الحمراء بجرعة (5–10) ملغم/ كغم من وزن الجسم، حيث أدى إعطاء المبيد الســـايبرمثرن إلى تثبيط إنزيم اللاكتيك ديهييدروجنيز معنويا بنسبة (9.5–4%) في البلازما وبنسبة 53% في كريات الدم الحمراء بجرعة (20–40) ملغم/ كغم من وزن الجسم.

Introduction

Inhibition of plasma or brain cholinesterase activity is a biomarker endpoint of exposure of wild birds to organophosphate and cypermethrin (1, 2, 3, 4, 5). Organophosphate insecticides are extensively used in public health, veterinary practice and agriculture (6, 7). These insecticides induce of enzyme cholinesterase with subsequent accumulation of acetylcholine at the nerve terminals and neuromuscular junctions (3, 8, 4). Birds are naturally deficient in cholinesterase activity in erythrocytes. Therefore the

diagnosis and monitoring of exposure to anticholinesterase insecticides rely on the measurement of plasma and erythrocytes cholinesterase activities in wild birds (1, 9, 10, 11, 12). The used an electrometric method for measurement of plasma and erythrocytes cholinesterase activity in several animal species. Application of an electrometric method for measuring plasma and tissue cholinesterase activities in four indigenous birds, The method of *in vivo* cholinesterase inhibition shown aspect intoxication by organophosphrous insecticide (13, 14, 15, 16).

Materials and Methods

- **Birds:** The birds Homer pigeon used in the present study were apparently healthy adults of both sexes. The birds were obtained from regions in which there were no activities of insecticide application for at least two months. The birds of each species were separately kept in captivity cages (80 × 60 × 70 cm) at about 25 °C with water and poultry feed for 14 days before experiments. Blood samples were collected using EDTA's (AFCO, Jordan) test tubes. Plasma was separated from blood by centrifugation at 3.000 rpm (Hettich, Germany) for 15 min.
- **An electrometric method for measurement of cholinesterase activity:** A pH 8.1 barbital-phosphate buffer solution (1.237 g sodium barbital, 0.163 g potassium dihydrogen phosphate and 35.07 g sodium chloride/L of distilled water) (15, 16). We determined cholinesterase activity in the plasma and RBCs samples by an electrometric method (17). The reaction mixture of the enzyme in a 10-ml vial contained 3 ml of the pH 8.1 barbital-phosphate buffer, 0.2 ml plasma or whole brain homogenate and 3 ml of distilled water. Initial pH of the mixture (pH 1) was measured by pH meter (Hanna, China), and then 0.12 ml of the aqueous solution of the substrate 7.5% acetylthiocholine iodide was added to the mixture as substrate which was incubated at 37 °C for 30 min. At the end of the incubation period, the pH of the reaction mixture (pH 2) was measured. The enzyme activity expressed as ΔpH/ 30 min was calculated as follows: Cholinesterase activity:

  $$(\Delta pH/30 \text{ min.}) = (pH\ 1 – pH\ 2) – \Delta\ pH \text{ of blank (15, 16).}$$
- **Lactic dehydrogenase activity by cypermethrin:** This method is based on colormetric method by using lactic dehydrogenase kit (Biolab, France) measured in computerized U. V. spectrophotometer (Labmed, USA) the kit prepare to zero conc. of cypermethrin as control and the residual cypermethrin activity in the mixture was measure as before.
- **Statistics:** The data were presented as means + SE and they were subjected to analysis of variance followed by the least significant difference test (18). The accepted level of statistically significant difference level was at $p<0.05$.

Results

- **Preliminary reference ChE activity:** A table 1 shows the normal ChE values, 95% confidence interval and related statistic for plasma and erythrocyte ChE activity of 12 homer pigeon. Preliminary reference values of the main cholinesterase activity (Δ pH/ 30 min**)** and confidence in the plasma were 0.197, respectively, and those of the erythrocyte were 0.943, respectively (Table 1). Erythrocyte ChE activity was significantly higher than that of the plasma.

Table (1) Preliminary reference cholinesterase activity (ΔPH/ 30 min) in the plasma and erythrocyte of homer pigeon

Sample	Plasma	Erythrocytes
Mean	0.298	0.492
Standard error	0.14	0.024
Standard deviation	0.683	0.983
Confidence	0.197	0.943

- ***In vivo* ChE inhibition:**

1. The insecticide dichlorvous significantly and in a dose-dependent inhibited plasma (28~39%) and erythrocyte 53% ChE activity *in vitro* (Table 2).

Table (2) *In vivo* inhibition of homer pigeon plasma and erythrocyte cholinesterase (ChE) by dichlorvous (mean ± SE)

Toxic dose mg\kg b.w.	Plasma ChE		Erythrocyte ChE	
	ΔPH / 30 min	% inhibition	ΔPH / 30 min	% inhibition
0 mg\kg b.w.	0.21 ± 0.018	0	0.39 ± 0.091	0
2.5mg\kg b.w.	0.05 ± 0.1	16	0.32 ± 0.022	19
5 mg\kg b.w.	0.03 ± 0.08*	28	0.37 ± 0.05	35
10 mg\kg b.w.	0.02 ± 0.41*	39	0.21 ± 0.6*	53

***significantly different from the respective control (0 mg\kg b.w.) p<0.05**

2. The insecticide diazinon significantly and in a dose -dependent inhibited plasma (33~38%) and erythrocyte 52% ChE activity *in vitro* (Table 3).

Table (3) *In vivo* inhibition of homer pigeon plasma and erythrocyte cholinesterase (ChE) by diazinon (mean ± SE)

Toxic dose mg\kg b.w.	Plasma ChE		Erythrocyte ChE	
	ΔPH / 30 min	% inhibition	ΔPH / 30 min	% inhibition
0 mg\kg b.w.	0.19 ± 0.033	0	0.25 ± 0.05	0
2.5mg\kg b.w.	0.12 ± 0.05	19	0.29 ± 0.31	21
5 mg\kg b.w.	0.09 ± 0.09*	33	0.2 ± 0.18	33
10 mg\kg b.w.	0.05 ± 0.022*	38	0.19 ± 0.096*	52

***significantly different from the respective control (0 mg\kg b.w.) p< 0.05.**

3. The insecticide cypermethrin significantly and in a dose -dependent inhibited plasma (4~9.5%) lactic dehydrogenase activity *in vitro* (Table 4).

Table (4) *In vivo* inhibition of homer pigeon of plasma lactic dehydrogenase by cypermethrin

Toxic dose mg\kg b.w.	Plasma ChE	
	Absorption	% inhibition
0 mg\kg b.w.	7.65 ± 0.27	0
20 mg\kg b.w.	3.94 ± 0.124*	4
40 mg\kg b.w.	2.91 ± 0.021*	9.5

***significantly different from the respective control (0 mg\kg b.w.) p<0.05.**

Discussion

The electrometric method is the common diagnosing organophosphate and carbamate poisoning in birds, It is therefore important to measure homer pigeon cholinesterase activities periodically to assess any environmental contamination by insecticides (9, 15, 20). The present electrometric technique for measurement of cholinesterase activity was used to assess poisoning induced by organophosphate and carbamate insecticides in chickens (15, 17, 21). The variations in the plasma and erythrocytes cholinesterase activities primarily in homer pigeon (17, 20, 22). The technique of *in vitro* cholinesterase inhibition by organophosphate insecticides can be used to assess the potential toxicity of anticholinesterase chemicals (17, 23). Organophosphate and carbamate insecticides in the avian species as well as in mammals primarily inhibit nervous system cholinesterases with subsequent development of nicotinic, muscarinic and central nervous toxicities (1, 4, 9). The inhibiton by cypermethrin *in vitro* effective to several enzymes present in plasma especially lactic dehydrogenase, it is necessary for aerobic metabolic path ways for conversion of lactic acid to pyrovate. (20) in addition it's effect on CYP450 in the liver. Their activities could be routinely measured using the described electrometric method to assess exposure of wild birds to agricultural insecticides polluting the environment.

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