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ENZYMATIC CHANGES IN SERUM AND BRAIN OF JAPANESE QUAIL EXPOSED TO CHLORPYRIFOS

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ABSTRACT

The activity of cholinesterase (ChE) in serum, acetylcholinesterase (AChE) in brain,as well as acid phosphatase (ACP), alkaline phosphatase (ALP), aspartate amino transferase (ASAT), and alanin amino transferase (ALAT) in serum and brain of the quail after oral exposure to various single doses of chlorpyrifos (0.1, 0.25, 0.5 and 1.0 LD50) for 24 hours or daily low dose (0.1 LD50) for three months was studied. Results revealed that exposure to the oral single tested doses caused a significant inhibition of both ChE in serum and AChE in brain after 24 hours. The daily exposure to the low dose (0.1 LD50) for three months resulted also in an inhibition of the activity in both serum and brain. The percentage of the inhibition of ChE in the blood serum following the acute poisoning reached about 90% of the control value, while that of AChE in the brain reached about 46%. ACP activities in both serum and brain were also inhibited. Significant decrease in ALP activity was observed in serum while it increased in the brain after acute and subchronic toxicity. In general, ASAT activity was inhibited significantly in the brain. On the other hand, the activity of ASAT in serum after 24 hours of exposure is generally increased while it decreased significantly after daily exposure for one, two, and three months. In contrast, the activity of ALAT increased significantly in both serum and brain. These changes in enzymes activity may reflect liver and / or kidney dysfunction and tissues damage.
Keywords: Chlorpyrifos, ChE, AChE, ACP, ALP, ASAT, ALAT, Japanese Quail.

INTRODUCTION

The organophosphorous compounds (OP) are still the most abundant class of Chemicals in large scale use as pesticides. Most of pesticides are not highly selective but are generally toxic to many non-target species including man and other desirable forms of life that co- inhibit the environment. The environmental contamination by pesticides due to their extensive and irresponsible use in agriculture and pests control has become a serious problem. However, the use of pesticides unfortunately is unavoidable to protect crops and increase farmers' income especially in the poor areas. The recent development of a biomarker based on the study of the biological response of organisms to pollutants has provided essential tools for the implementation of programmes for contamination monitoring (Peakall and Shugart, 1991)

Measuring of AChE is probably the best general indicator of serious OP and Carbamate (C) pesticides pollution (Cook et al., 1976). Determination of brain AChE activity is widely used to diagnose OP and C poisoning (Hart, 1993). Taking into consideration that the activity of AChE is reduced to less than 50% of the normal level by OP and C, this degree has been regarded as a good indicator of poisoning (Coppage and Mathews, 1974, Westlake et al., 1981 a, b). The levels of tissue – specific enzymes circulating in the plasma

have been studied to monitor the sublethal effects of a number of pesticides in quail (Westlake *et al.,* 1981 b). They demonstrated that measurement of plasma and brain esterase levels are of value in the diagnosis of organophosphate poisoning in quail. Ludke et al. (1975) found that analysis of brain ChE activity proved to be reliable for diagnosing and monitoring effects of selected ChE inhibitors in birds. Also, Hill (1989) showed that blood plasma cholinesterase (ChE) activity of quail is a sensitive indicator of exposure to organophosphorous insecticides. Prijono and Leighton (1991) reported that activity of AChE in brain of quail decreased after lethal intoxication with Diazinon.

The OP toxicity was always indicated by a significant depression of serum and / or red blood cell cholinesterase activities (Vasilic et al., 1992). Similarly, Gomes *et al.* (1999) reported that chronic exposure to organophosphorous pesticides significantly inhibits acetylcholinesterase (AChE) activity in mice. Results of Monnet et al. (2000) indicated that inhibition of AChE remains the most sensitive macromolecular target of OP exposure, since toxic effects were found at concentrations in which AChE was inhibited. Wilson et al. (2001) observed that exposure of California quail to OP insecticides caused an immediately decline in ChE levels to 61% compared to control values. Also, Dyer et al. (2001) observed significant depress of serum ChE activity in pest control operators exposed to chlopyrifos.

Measurement of the phosphatase enzymes is important since acid phosphatase (ACP) was the first tumour marker to be measured in the blood (Douglas, 1963) and it is a marker enzyme for lysosomes, thus related to general cell metabolism. Alkaline phosphatase (ALP) is a sensitive biomarker to metabolic salts since it is a membrane – bound enzyme related to the transport of various metabolites, it is particularly abundant in bile canaliculus and is used to diagnose liver dysfunction in mammals (Farag and Salman, 1989). Saigal et al. (1982) found that adult rats exposed to a single oral doses of some pesticides exhibited significant impairment in acid and alkaline phosphatases activities within one hour of dosing. Soliman *et al.* (1983) reported that sheep treated with single oral dose of liptophos showed a significant decrease in the levels of ALP in the blood serum in the first week after treatment and came to within the control levels four weeks after treatment. Also, prolonged exposure (8 weeks) of mice to chlorpyrifos indicated that the activity of serum ALP had increased non – significantly (Gomes et al., 1999). In addition, El – Demerdash (2001) stated that rats exposed to oxidative stress of Hg or Se exhibited a significant decrease in both acid and alkaline phosphatase activities in the blood plasma.

Monitoring of liver enzymes leakage in the blood has proved to be a very useful tool in hepatotoxic studies (Kaplan *et al.*, 1988). Mikhail et al. (1979) studied the influence of acute poisoning with chlorpyrifos on serum enzymes in albino rats. They showed that injection of chlorpyrifos in a dose of 0.5 LD50 resulted in a significant increase in serum aspartat amino transferase (ASAT) and alanine amino transferase (ALAT). Westlake *et al.* (1981b) showed that plasma ASAT was elevated in quail surviving carbophenothion and pirimiphos methyl treatment at 24 hours. Also, Mohamed et al. (1990) observed a

significant increase in the activity of ASAT in serum of Nubian goats after exposure to (75 – 1200 mg/Kg) of chlorpyrifose.

ASAT and ALAT activities were decreased in the blood serum of fish exposed to low dose (0.1 LC50) of pirimiphos – methyl during the first two days of exposure and then increased over that of control. On contrary, at the highest concentrations (0.4, 0.8 and 1.0 LC50) the activities increased as compared to that of control (El-Gougary et al., 1999). Prolonged exposure of mice to some organophosphorous pesticides resulted in a significant increase in both ASAT and ALAT (Gomes et al., 1999). In addition, exposure to heavy metals also lead to significant increase in the activities of both ASAT and ALAT in the blood serum (El – Demerdash, 2001 and Adham et al., 2002) However, Enan et al. (1982) reported that acute and chronic exposure of white rats to some organophosphorous insecticides exhibited significant inhibition in the activity of ASAT.

In this study the effects of short and long term exposure of Japanese quail to chlorpyrifos on the activities of some enzymes in serum and brain were investigated.

MATERIALS AND METHODS

1. Tested pesticide:

Chlorpyrifos (Dursban) [O,O - diethyl – O - (3,5,6-trichloro – 2 - pyridyl) phosphorothioate] was the tested pesticide. It was obtained in the form of commercial product from the local market.

2. Tested animals:

Male japanese quail (Coturnix coturnix japonica) 120 – 160 gm weight were used. The birds were kept for acclimatization in the laboratory for one week before starting the experiments.

3. Toxicological studies:

Value of the LD50 of chlorpyrifos used in this study was 680 mg /Kg body weight as reported previously by Ibrahim and El-Zahrani (2007).

Two experiments were carried out as follow:

a) Effect of exposure to sublethal oral single doses (0.1, 0.25, 0.5 and 1.0 LD50) of the tested insecticide for 24 hours was tested to show the acute effect.

b) Effect of daily oral exposure to the low dose (0.1 LD50) of the tested insecticide orally for one, two and three months was tested to show the subchronic effect.

Ten birds were used for each treatment; the treated birds were given the tested doses orally as a solution in 2 ml. corn oil for each bird by gastric tube while the birds of the control groups received corn oil only.

Blood sampling:

Blood samples were collected from the heart directly (Johnson, 1981) serum was obtained by cooling centrifugation at 1400 xg for 10 minutes and then stored in deep freeze at -18°C for different analysis.

Brain sampling:

Brains were removed, chilled on ice and homogenized with 20 volumes of saline solution 0.9%. The homogenates were centrifuged at 1400 xg for 10 minutes and the supernatants were stored for different analysis.

Samples from six random animals were taken for rather blood or brain analysis for each treatment.

Measurements of the enzymes:

Standard kits were used to determine the activities of the following enzymes in both serum and brain of the tested birds:

Cholinesterase (ChE) in serum and acetylcholinesterase (AChE) in brain (Knedel and Bottger, 1967), ACP (Tietz, 1995), ALP (Henry, 1974), ASAT (Saris, 1978) and ALAT (Greiling and Gressner,1995)

Data analysis:

Statistical analysis was carried out according to Snedecor and Cochran (1969) using the SPSS software. Duncan's multiple range test was used to determine the specific differences between treatments.

RESULTS

The activities of all the tested enzymes in serum (U/L) are shown in Tables 1 and 2, while in brains (expressed as percentages of that of the control) are illustrated in Figures 1 and 2.

ChE activity in serum of the treated birds after 24 hours of administration of the tested single doses were strongly inhibited comparing to that of the control birds. The activity was decreased from 4007.2 ± 473.0 in the control to 368.6 ± 40.0 – 434.8 ± 41.4 in the treated birds. Results indicated also that there were no significant differences between the values in the different tested doses (Table 1). Also, daily exposure to 0.1 LD50 for one, two and three months caused a significant decrease in the activity of ChE as shown in Table (2).

AChE activity in the brain was inhibited significantly after exposure to the single tested doses for 24 hours or the low daily dose for three months. Activities expressed as percentages of the control value were ranged between 53.2% and 84.8% (Fig. 1 and 2).

Acid phosphatase activity in serum of quail after 24 hours of administration of the doses equal to; 0.1, 0.25, and 0.50 LD50 showed significant decrease comparing to that of the control. The most obvious degree of decrement was recorded in case of using 0.25 LD50 dose; it was 16.4 ± 1.2 and 0.6 ± 0.3 U/L in control and treated birds, respectively as shown in Table (1). In contrast, the tested birds exposed to the LD50 dose recorded a sudden

highly significant increase in the activity of that enzyme as compared to control (45.6 ± 3.9 U/L). Also, ACP activities in serum of the tested birds after daily administration of the low dose (0.1 LD50) for one, two and three months showed a highly significant decrease throughout the experimental period (Table 2).

The activity of ACP enzyme in the brain tissue of the tested birds showed also an obvious significant decrease comparing to that of

control following exposure to the different single tested doses for 24 hours. The Same trend was observed in the birds exposed to the low dose (0.1 LD50) daily for the long periods (one, two and three months). The ACP activities in the brain (expressed as percentage of the control) of the treated birds were ranged between about 9.0% and 16.3% (Fig. 1 and 2).

Table (1): Enzymes activity in serum (U/L) after 24 hours of exposure to different oral single doses of chlorpyrifos

Dose (LD50)	ChE	ACP	ALP	ASAT	ALAT
0	4007.2±473.0a	16.4±1.2b	1776.8±142.5a	830.0±38.8c	33.8±4.2e
0.1	434.8±41.4b	4.9±0.8c	464.2±87.3d	762.6±77.4c	50.0±8.1d
0.25	428.3±4.9b	0.6±0.3e	580.2±62.6c	1625.8±98.0a	92.6±6.1b
0.50	396.9±12.3b	2.0±0.1d	755.8±70.6b	894.2±113.0c	154.0±22.0a
1.0	368.6±40.0b	45.6±3.9a	596.2±13.5c	904.2±121.8b	70.2±5.0c

Different superscripts differ significantly at < 0.05

Table (2): Enzymes activity in serum (U/L) following daily exposure of 0.1 LD50 chlorpyrifos for one, two and three months

Duration	ChE		ACP		ALP		ASAT		ALAT	
	C	T	C	T	C	T	C	T	C	T
1 month	3508± 384c	598.7± 43.7b	16.4± 0.7a	1.7 ± 0.7c	1762.5± 83.0a	653.0± 159.5b	784.8± 83.8a	660.0± 72.4b	39.8± 4.5b	70.2± 21.8a
2 months	3669± 259c	750.6± 80.6ab	17.1± 0.4a	2.2 ± 0.5c	1767.0± 52.5a	328.0± 36.4c	867.8± 40.2a	609.8± 64.8b	42.5± 1.8b	46.8± 5.4ab
3 months	4053± 681c	775.8± 67.7a	14.2± 0.5b	1.6± 0.3c	1851.2± 31.5a	148.8± 30.8d	661.5± 27.3a	265.2± 63.8c	41.3± 2.9b	47.0± 3.8b

Different superscripts differ significantly at < 0.05
C: Control birds, T: Treated birds

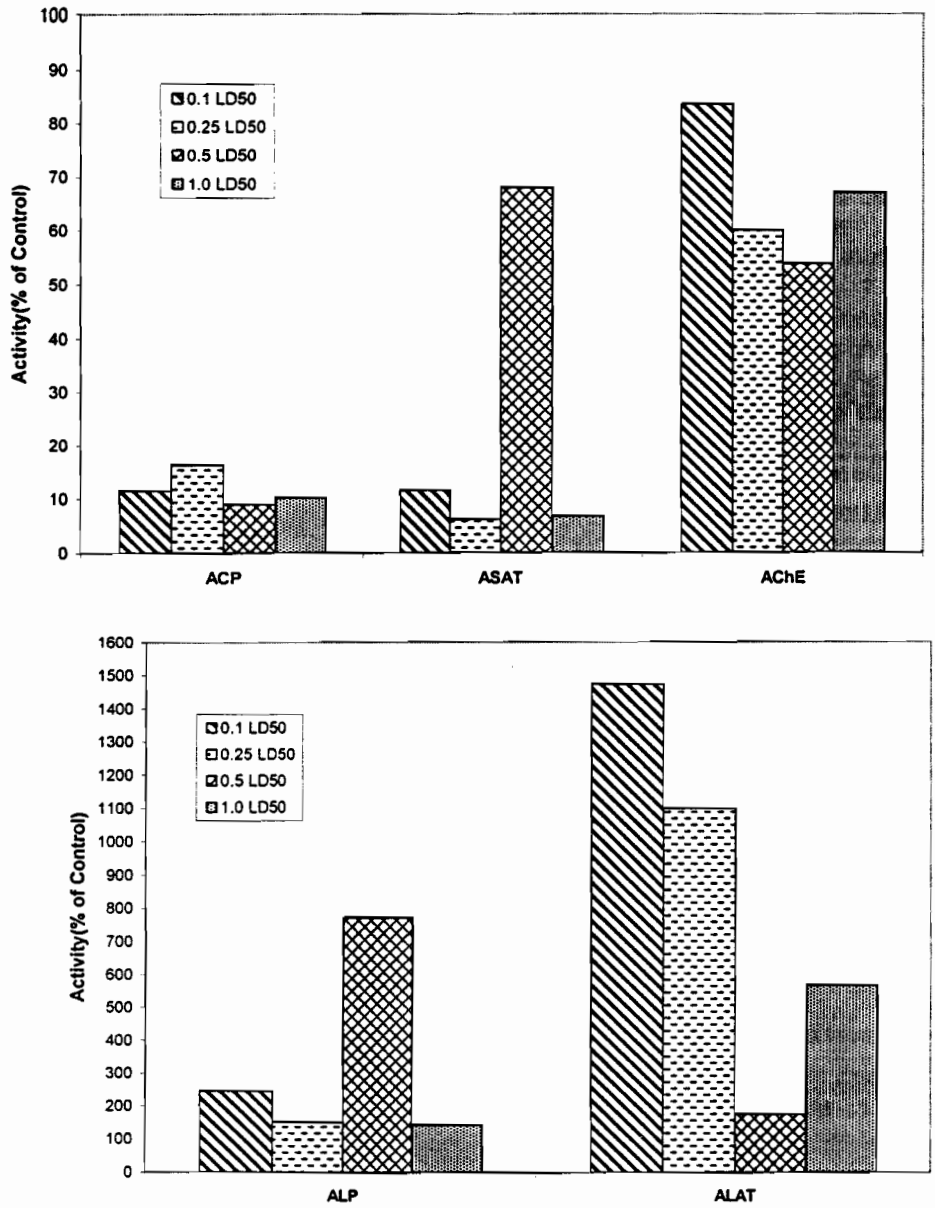

Figure 1: Activity (as % of control) of ACP, ASAT and AChE (top) and for ALP and ALAT (bottom) in brain after 24 hr. of exposure to the single tested doses of Chlorpyrifos.

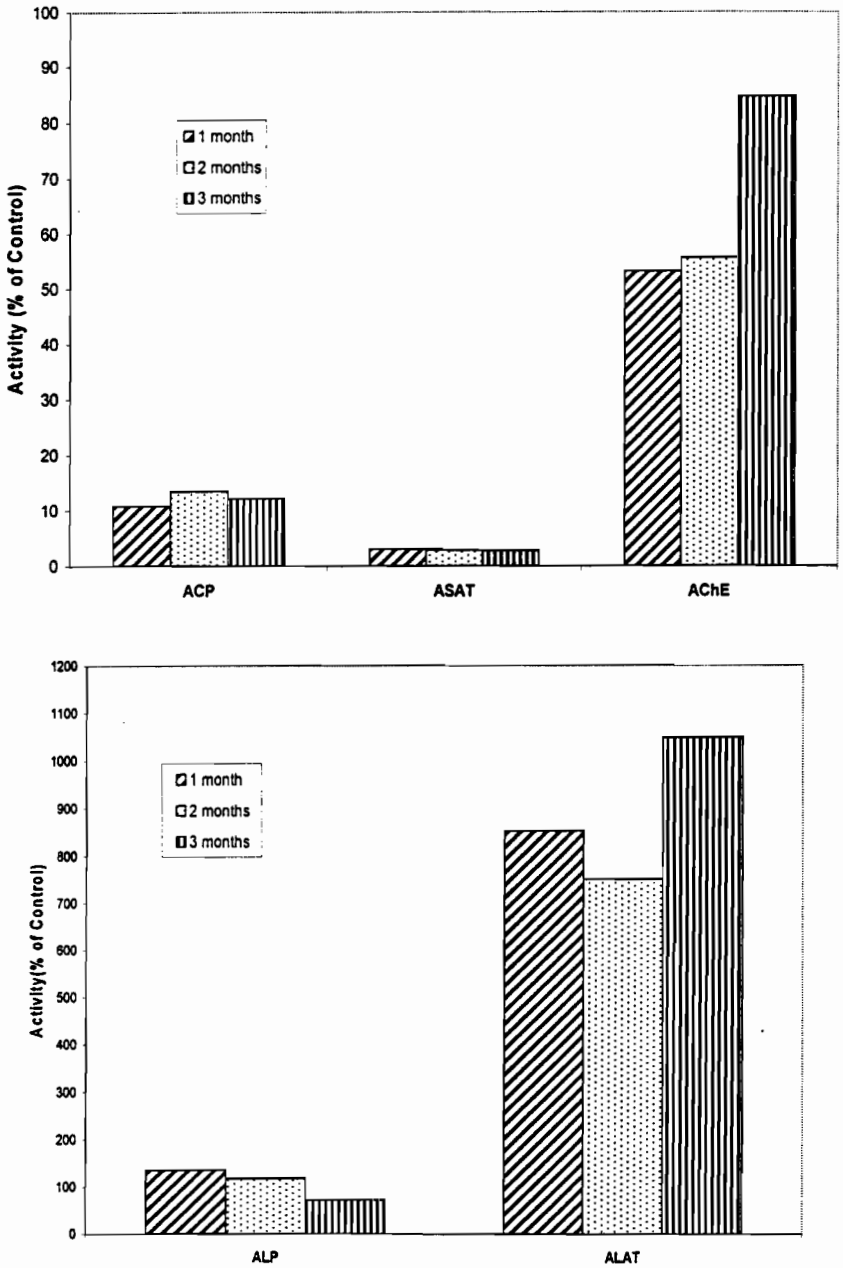

Figure 2: Activity (as % of control) of ACP, ASAT and AChE (top) and for ALP and ALAT (bottom) in brain, following the daily exposure to 0.1 LD50 dose of Chlorpyrifos for 1, 2 and 3 months.

Concerning ALP activity in the blood serum of the treated birds exposed to the oral single tested doses for 24 hours and the daily low dose (0.1 LD50) for three months, data showed that the activity decreased significantly as compared to that of the control birds. The value was 1776.8 ± 142.5 U/L in control and it ranged between 464.2 ± 87.3 and 755.8 ± 70.6 U/L in the treated birds after 24 hours of exposure (Table 1), while the values ranged between 1762.5 ± 83 and 1851.2 ± 31.5 U/L in the control birds and between 148.8 ± 30.8 and 653.0 ± 159.5 U/L in the treated birds for one, two and three months (Table 2). On the other hand, ALP activity in the brain showed a significant increase in the treated birds after 24 hours from the oral single doses exposure, the highest increment was observed in case of exposure to 0.5 LD50 dose. The percentages of the increment were ranged between 143.9% and 771.5% relative to the control value (Fig.1). However, administration of the low daily dose (0.1 LD50) for three months caused a slight increase during the whole period of the experiment (Fig. 2).

Regarding ASAT activity in the blood serum after 24 hours of the single doses administration, irregular dose – independent trend was noticed since the values was significantly increased in case of exposure to 0.25 and 1.0 LD50 doses ,while no significant changes were observed in case of 0.1 and 0.5 LD50 (Table 1). In contrast, exposure to the low daily dose (0.1 LD50) leads to a significant decrease in ASAT activity after one, two and three months as illustrated in Table (2).

The activity of ASAT in the brain was reduced significantly after 24 hours of exposure to all the single tested doses. The activities were: 11.5, 6.2, 68.0 and 6.8% relative to that of control in case of 0.1, 0.25, 0.5 and 1.0 LD50 doses, respectively (Fig. 1). Also, highly significant decrease was recorded following administration of 0.1 LD50 dose daily for one, two and three months (Fig. 2).

ALAT activity in serum of the treated birds after 24 hours of exposure to the different oral single tested doses exhibited a significant elevation than control. This elevation was dose – dependent up to the dose equal to 0.5 LD50 (Table 1). In addition, the daily exposure to the low dose (0.1 LD50) caused also a significant increase after one month while the increase after two and three months of daily exposure was insignificant as illustrated in Table (2).

Similarly, the activity of ALAT in the brain increased significantly after exposure of the birds to the tested doses either for 24 hours or after one, two and three months of daily exposure. The percentages of the activities were ranged between 175.7% to 1472% and between 750.7% to 1048.2% relative to that of control in the short and long periods experiments, respectively (Fig. 1 and 2).

DISCUSSION

The measurement of different esterases and other tissue specific enzymes in plasma and brain of avian species may be useful in monitoring the sublethal effects of pesticides on wild life.

In the present study, ChE activity was strongly inhibited in both serum and brain of the treated birds following acute exposure to chlorpyrifos. This inhibition reached about 90%, 46% of the control values in serum and brain respectively.

A similar trend of inhibition has also been observed in serum and brain after a long – term exposure. These results are in agreement with those previously reported by Westlake *et al.* (1981b), Gomes *et al.* (1999) and Wilson et al. (2001). It is known that ChE inhibition caused by OP compounds is attributable to phosphorylation of the esteratic site of the enzyme by the oxygenated metabolites of these compounds. Therefore, the phosphorathionate (P = S) pesticides such as chlorpyrifos are converted into their oxygen analogue "oxon" (P = O), through activation pathways, before becoming strongly irreversible inhibitors of ChE to manifest their biological activity (Eto, 1974 and Bocquene et al., 1995).

In general, measurement of ChE inhibition in brain tissue or blood serum of birds can be used as an indicator of exposure to ChE – inhibiting insecticides (Ludke, *et al.,* 1975, Hill and Fleming, 1982 and Wilson et al., 1991).

Acid Phosphatase (ACP) activity was significantly inhibited in both serum and brain of the tested birds after acute and subchronic exposure to all the tested doses regimen except in case of serum of birds exposed to the highest single dose (1.0 LD50) which exhibited a significant increase 24 hours after treatment. It was suggested that the observed increase could be attributed to haemolysis or kidney dysfunction (Bernard and Henry, 1996).

The observed decline in the Alkaline Phosphatase (ALP) activity in serum of the treated birds 24 hours after treatment with the oral single tested doses and the lower oral dose (0.1 LD50) given daily for three months is in agreement with data previously reported by Cervelli et al. (1978). Also, Saigal et al. (1982) found that exposure of male adult albino rats to single oral doses of some pesticides lead to significant impairment in acid and alkaline phophatase activities in different tissues within one hour of dosing. Soliman et al. (1983) also found that acute exposure of sheep to leptophos decreased ALP levels in serum.

On the other hand, ALP activity in the brain of the treated birds was significantly increased 24 hours after administration of all the tested oral single doses. Similar results were obtained in brain of mercuric chloride treated mice (Mehra and Kanwar, 1986). Also, the activity of this enzyme was increased in the liver after acute poisoning of rats with organophosphate insecticides (Murphy, 1966). He concluded that poisoning by chemicals might produce metabolism alteration that were mediated through the pituitary – adrenal system, and occurred in tissue remote from the primary sites of toxic action.

In this study, the increase in serum ASAT activity after acute exposure to some of the tested doses is in agreement with that reported by many other workers (Westlake *et al.,* 1981a, b; Mohamed et al., 1990 and El-Gougary *et al.,* 1999). They related this elevation to liver dysfunction and tissue damage caused by chemical exposure.

On the other hand, significant inhibition in ASAT activity was observed in the brain after acute and subchronic exposure. In addition, there was a

significant inhibition in ASAT activity in the blood serum after the multiple dose exposure to the tested insecticide. This result is in agreement with that previously reported by Enan *et al.* (1982). Adham *et al.* (2002) explained ASAT inhibition on basis of the adverse effects on the enzyme precursors in the liver resulting in slowing biochemical turnover.

The general significant increase in ALAT activity observed in serum and brain after acute and chronic poisoning is in agreement with the results previously obtained by Mikhail et al. (1979); Gomes et al. (1999) and El-Gougary et al. (1999). This elevation in ALAT activity may be attributed to the leakage of this enzyme from the damaged tissues into the surrounding body fluids (William, 1997).

It can be concluded from the above mentioned results of the current study that chlorpyrifos beside its main cholinesterase inhibitory action has also different physiological effects resulted in enzymatic changes. These effects and changes are dose and exposure duration dependent. Although our results agree in general with what has been published previously, our data also contradict with some other results to some extent. This diversity could be attributed to different factors as the type of the tested insecticide, dosing regimen, animal species (Chambers and Carr, 1995), and sex (Hill, 1989).

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التغيرات الانزيمية فى مصل ومخ طيور السمان المعرضة لمبيد الكلوربيروفوس

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اجريت هذه الدراسة لمعرفة التغيرات فى نشاط انزيم الكولين استيريز (ChE) فى المصل والاستيل كولين استيريز (AChE) فى المخ وكذلك انزيمات الفوسفاتيز الحامضى (ACP) والقاعدى (ALP) وانزيم ALAT, ASAT فى مصل ومخ طيور السمان، بعد إعطائها جرعة واحدة عن طريق الفم من مبيد الكلوربيروفوس بتركيزات مختلفة (٠,١ ، ٠,٢٥ ، ٠,٥ ، ١,٠) من قيم LD50 لمدة ٢٤ ساعه او اعطائها جرعة منخفضة (٠,١ من قيمة LD50) يوميا لمدة ٣ شهور.

اوضحت الدراسة ان اعطاء جرعة واحدة بتركيزات مختلفة ادت الى نقص نشاط انزيمى AChE, ChE بعد ٢٤ ساعة، كما حدث تثبيط لهذه الانزيمات بعد اعطاء الجرعة المنخفضة (٠,١ من قيمة LD50)، لمدة شهر وشهرين وثلاثة شهور حيث وصلت نسبة التثبيط بعد ٢٤ ساعة من تعاطى الجرعات الى حوالى ٩٠% فى الدم ، ٤٦% فى المخ بالمقارنة بالمجموعة الضابطة (control).

ولقد بينت النتائج ايضا ان نشاط انزيم الفوسفاتيز الحامضى فى كل من الدم والمخ قد انخفض، وكذلك حدث تثبيط لنشاط انزيم الفوسفاتيز القاعدى فى الدم بينما ارتفع نشاطه فى المخ نتيجة التعرض الحاد والمزمن للمبيد. كما انخفض نشاط انزيم ASAT بصفة عامة فى المخ، كذلك ادى التعرض المزمن للمبيد لمدة ٣ شهور الى انخفاض نشاط هذا الانزيم فى المصل، بينما ازداد نشاط الانزيم فى المصل فى حالة التعرض الحاد لتركيزات مختلفة من المبيد لمدة ٢٤ ساعة. ولقد اظهرت النتائج ازدياد نشاط انزيم ALAT فى الدم والمخ بعد التعرض الحاد والمزمن للمبيد مقارنة بالمجموعة الضابطة (control). وتعكس هذه التغيرات الانزيمية حدوث خلل فى وظائف الكبد والكلى وتهتك الانسجة.