J. Agric. Sci. Mansoura Univ., 32 (5): 3759 - 3770, 2007

EFFECT OF CHLORPYRIFOS TOXICITY ON SOME BIOCHEMICAL PARAMETERS IN SERUM OF QUAIL (*Coturnix coturnix japonica*).

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ABSTRACT

The present study was conducted to evaluate the acute and subchronic effect of chlorpyrifos on some biochemical parameters in the blood serum of exposed quail. The results illustrated that exposure to sublethal single doses (0.1, 0.25, 0.5 and 1.0 LD_{50}) for 24 hours lead to an elevation of haemoglobin, ammonia and glucose (to some extent), while bilirubin, cholesterol, phospholipids, urea N and uric acid content decreased. In contrast, total protein and creatinine showed an irregular trends. Inorganic P was significantly increased at the highest dose (LD_{50} value) while triglycerides content increased significantly only in case of administration of the lowest dose (0.1 LD_{50}). Concerning the effect of daily administration of the low sublethal dose (0.1 LD_{50}) for 3 months, it was found that haemoglobin, ammonia, total protein and triglycrides increased, while bilirubin decreased in the treated birds during the whole period of the experiment. On the other hand, cholesterol, phospholipids, creatinine and uric acid did not change significantly. The results indicated also that glucose, inorganic P, urea – N changed irregularly. These changes in the blood constituents may be explained on the basis of liver and / or kidney dysfunction, tissues damage in addition to enhancement or inhibition of the activity of some regulating enzymes.
Keywords: Quail, Chlorpyrifos, Oral administration, Serum biochemical parameters.

INTRODUCTION

It is well established that various types of pesticides with special reference to organophosphorous compounds are extensively and widely used in agriculture and homes. These toxic compounds enter the body through different routes including, inhalation, absorption via the skin, or ingestion of food or contaminated water (British Medical Association, 1992 and Health & Safety Executive, 1993). As a result, many environmental problems were appeared not only for human but also for plants and animals. These problems were also extended to include soil, air and different water bodies.

Chlorpyrifos (Dursban) is an organophosphate compound marketed in numerous formulations as plant or livestock insecticide and it is used for control of insects. Aerial application is very common and consequently pollution of water resources and contamination of vegetables and other plants are likely to occur and threaten both human and animal health.

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). The biochemical measurements are effective means for studying the impact of such pesticides on the exposed animals.

Abdel Aziz (2000) found that the 0.1 of LD_{50}/Kg daily interaperitoneal injection of some pesticides (Fenpropathrin, Thionex and Thiocyclam) to adult male albino rats for 5 days showed a general decrease in RBCs count and Hb content. Exposure of freshwater field crab *Oziotelphusa senex senex* to Chlorpyrifos caused an increase in the haemolymph ammonia in both lethal and sublethal concentrations (Radhakrishnaiah *et al.*, 1995). Levels of bilirubin in both plasma and serum of sheep were slightly decreased after 24 hours when animals exposed to a single oral dose (500 mg/kg) of Tri-Ortho-Cresyl Phosphate (TOCP) (Soliman *et al.*, 1983).

Jimenez and Pocsidio (1994) observed that levels of glucose decreased when bolti fish were exposed to some organophosphrus insecticides. On the other hand, Soliman *et al.* (1983) found that the levels of glucose were slightly increased in the plasma of sheep, but it did not affect serum. In addition, Yassin (1998) reported that glucose content of rabbits blood serum responded differently to the treatments by some insecticides.

Soliman *et al.* (1983) found also that exposure of sheep to single dose of leptophose leads to significant increase of total protein in sheep serum. In contrast, El-Gougary *et al.* (1999) showed that protein level was reduced in the blood serum of bolti fish (*Orechromus niloticus*) when it exposed to pirimiphos methyl. The process of protein reduction was correlated to the increase of tested concentration. They reported also that such decrease slowed down with the increase of exposure period to reach normal levels.

Generally, several studies indicated that levels of cholesterol in serum, decreased after exposure to different organophosphorous insecticides (Yassin, 1998 and Abdel Aziz, 2000). According to Soliman *et al.* (1983) and Abdel Aziz (2000), exposure of different animals to some organophosphorous pesticides and compounds lead to elevation in triglycerides content. On the other hand, Yassin (1998) reported that triglycerides content of rabbit blood serum responded differently to the treatment by different organophosphorous insecticides. Also, Makhija and Pawar (1977) reported that the phospholipids increased obviously in rats after treatment with some organophosphorous and carbamate pesticides (malathion and isopropoxy phenyl – N – methyl carbamate). Soliman *et al.* (1983) found that inorganic phosphate was significantly decreased in plasma and serum of sheep treated with TOCP after 24 hours.

The administration of sublethal doses of some organophosphorous insecticides for few days raised up the concentration of urea, uric acid, and creatinine (Yassin, 1998 and Abdel Aziz, 2000). However, El-Sebae *et al.* (1981) reported that the effect of insecticides administration on urea content depends on the sex of tested animals and the different tested toxicants. Some of the tested insecticides raised up the urea content while others caused a general decrease.

The present study was conducted to detect and monitor the effects of chlorpyrifos on some biochemical parameters in the blood serum of quail. This may provide not only early warning about health of a different species but also degradation in environmental quality.

MATERIALS AND METHODS

I) Experimental pesticide:

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

II) Experimental animals:

Mail quail (*Coturnix coturnix japonica*) 120 – 160 gm weight were selected for this study since it is one of the most popular avian species in Saudi Arabia. The birds were left to acclimate with laboratory conditions for one week before conducting the experiments.

III) Toxicological studies:

Three experiments were carried out as follow:

1) Determination of LD_{50} value:

The lethal dose (LD) was determined experimentally by testing initially three different doses; 10,100 and 1000 mg/kg body weight, after which the percentage of mortality was recorded after 24 hours of oral administration. From the above mentioned experiment, the lethal dose which caused death of all the experimental animals and the other doses which did not cause any death were determined. Therefore, the main experiment was designed to compute the value of LD_{50} by using 5 different concentrations between the two previously mentioned concentrations and count the percentage of mortality for each dose separately after 24 hours of administration, from this experimental test, the value of LD_{50} – the dose which cause death for 50% of the birds within 24 hours – was computed.

2) Short-term effect of sublethal doses:

Animal groups were exposed to four doses (0.1, 0.25, and 0.5 of the LD_{50} as well as the LD_{50} dose) for 24 hours to show the acute effect.

3) Long-term effect of the sublethal doses:

Animals were exposed daily to 0.1 LD_{50} for three months. The samples were collected after one, two, and three months to show the subchronic effect.

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

Blood sampling:

Six animals were taken randomly from each treatment. Blood samples of the control and treated birds were collected from the heart directly (Johnson, 1981) then clear serum samples were separated by cooling centrifugation at 1400 xg for 10 minutes and then stored in deep freeze at -18°C for different analysis.

Measurements of biochemical parameters:

Haemoglobin (Hb) was determined in blood samples using Coulter counter. Serum samples were analyzed calorimetrically for: ammonia (Van Anken and Schiporst, 1974), bilirubin (Bakerman, 1983), glucose (Trinder, 1969), total protein (Flack and Woollen, 1984), cholesterol (Stadtman, 1957), triglycerides (Fossati and Prencipe, 1982), phospholipids (Takeyaman *et al.*,

1977), inorganic phosphorus (Daly and Ertingshausen, 1972), creatinine (Widmann and Frances, 1974), urea nitrogen (Talke and Schubert, 1965), and uric acid (Fossati *et al.*, 1980).

Statistical analysis:

Mean, standard deviation and standard error were determined according to Turner (1970) using SPSS software. Duncan's multiple range test was used to determine the specific differences between treatments.

RESULTS

In the present study, the experimental test for determination the LD_{50} value of chlorpyrifos in quail revealed that this value was calculated to be 680 mg/kg body weight. Results of all the different tested biochemical parameters are illustrated in Tables (1) and (2) and Figs. (1) and (2).

Haemoglobin (Hb) levels in blood of the tested birds were generally significantly higher in the exposed groups as compared to control. This increase is more obvious after oral administration with o.1 LD_{50} for long periods (1 – 3 months). The values ranged from 11.7 to 12.4 in the control and from 20.3 to 21.4 g/dL in the treated birds as shown in Figs. (1) and (2).

The levels of ammonia in serum of the experimental animals were significantly increased after 24 hours subsequent to administration of both 0.1, 0.25 and 0,5 LD_{50}, in comparison to the control. The use of LD_{50} dose on the other side showed no significant effect on levels of ammonia. Also, administration of the low dose; 0.1 LD_{50} daily for one, two and three months resulted in significant increase on the levels of ammonia in serum compared to control, in which the highest value was recorded after two months.

Table (1): Biochemical Parameters Concentration in Blood Serum of Quail After 24 Hours of Chlorpyrifos Oral Administration.

Parameters	Dose (LD_{50})				
	Control	0.10	0.25	0.50	1.0
Ammonia (µmol/L)	781^{d}±130	2104^{a}±141	1242^{c}±137	1480^{b}±52	700^{d}±106
Bilirubin (µmol/L)	17.8^{a}±2.2	9.3^{b}±1.1	6.8^{c}±1.6	2.4^{d}±0.6	1.1^{d}±0.4
Glucose (mmol/L)	25.5^{bc}±1.0	22.3^{c}±1.6	27.7^{ab}±3.0	26.1^{bc}±1.8	31.4^{a}±1.8
Total Protein (g/L)	24.8^{c}±1.3	31.4^{a}±1.7	27.6^{b}±2.3	3.2^{d}±1.1	2.2^{d}±0.3
Cholesterol (mmol/L)	41^{a}±0.5	3.3^{b}±0.5	3.1^{b}±0.6	2.5^{bc}±0.4	1.8^{c}±0.4
Triglycerides (mmol/L)	2.8^{b}±0.6	8.3^{a}±0.7	2.5^{b}±0.3	2.5^{b}±0.9	2.3^{b}±0.5
Phospholipids (mmol/L)	5933^{a}±399	5089^{bc}±418	5205^{b}±530	4436^{cd}±424	3727^{d}±451
Inorganic P (mmol/L)	2.7^{b}±0.5	2.7^{b}±0.3	2.3^{b}±0.2	2.5^{b}±0.5	4.9^{a}±0.4
Creatinine (µmol/L)	23.0^{b}±2.6	23.6^{b}±1.7	31.6^{a}±1.8	6.4^{c}±0.6	4.8^{c}±1.5
Urea-N (mmol/L)	2.6^{a}±0.05	1.7^{ab}±0.50	1.7^{ab}±0.30	0.6^{c}±0.20	1.4^{bc}±0.60
Uric acid (µmol/L)	1191^{a}±122	557^{c}±46	338^{d}±27	320^{d}±11	720^{b}±79

Different superscripts differ significantly at p<0.05

Fig.(1) :In Vivo Oral Effect Of Chlorpyrifos On Quail Blood Haemoglobin (24 hours after treatment)

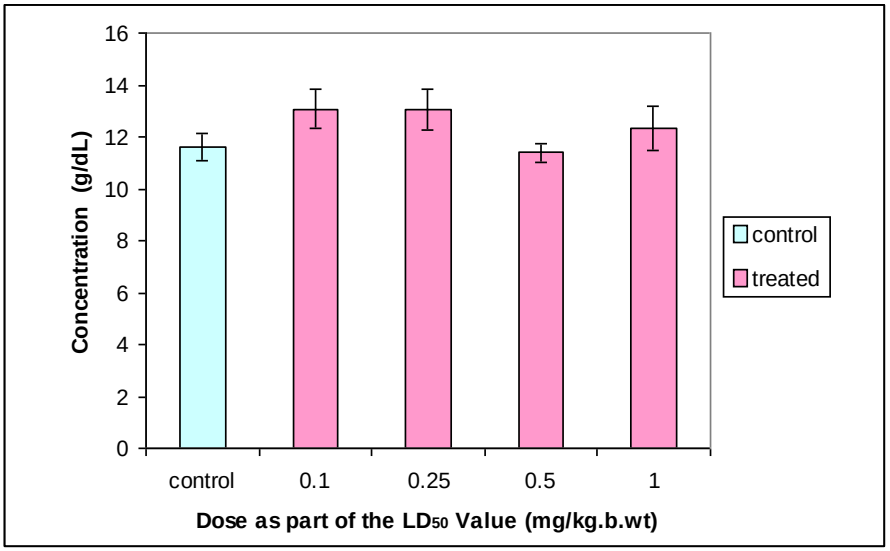
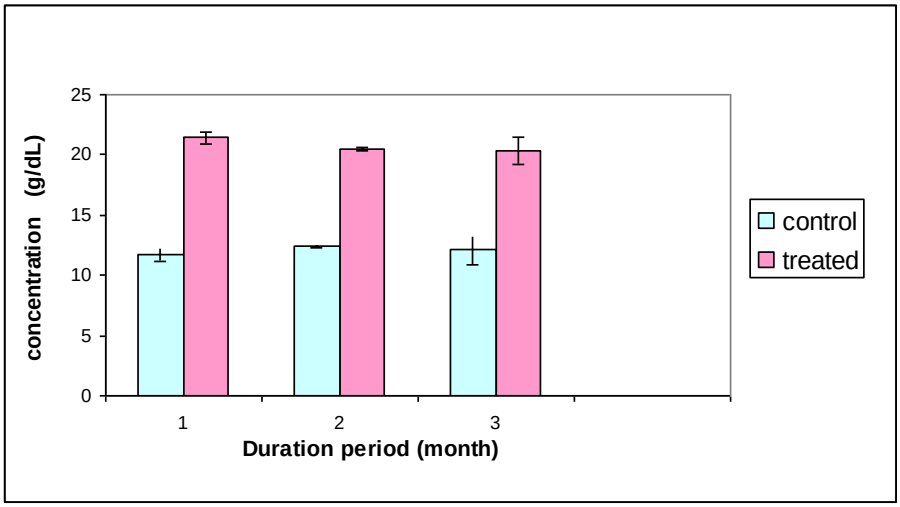


Fig. (2): In Vivo Oral Effect Of Duration Period Of Chlorpyrifose (0.1 LD_{50}) On Quail Blood Haemoglobin (1month, 2months, 3months after treatment)



Obtained data indicate that concentrations of bilirubin in serum was significantly decreased after 24 hours of 0.1 ,0.25 ,0.50 and 1.0 LD_{50} administration as compared to the control values. Levels of serum bilirubin were decreased with increasing the used dose, they were 17.8 ± 2.2, 9.3 ±

1.1, 6.8 ± 1.6, 2.4 ± 0.6 and 1.1 ± 0.4 µmol/L for control, 0.1, 0.25, 0.5 and 1.0 LD_{50} respectively. Also, bilirubin content in serum were significantly decreased after one and three months of daily oral administration of the low dose (0.1 LD_{50}), while administration of the tested insecticide daily for two months did not show any significant differences. Generally, there was an elevation in the levels of glucose in blood serum of the treated birds, this elevation was significant only after administration of a dose equivalent to 1.0 LD_{50} for 24 hours and 0.1 LD_{50} for two months.

As shown in Table (1), the levels of total protein in serum of the treated birds after 24 hours of administration of both doses; 0.1 and 0.25 LD_{50} value showed a significant increase as compared to control (31.4 ± 1.7, 27.6 ± 2.3 and 24.8 ± 1.3 g/L for 0.1, 0.25 LD_{50} and the control, respectively), then the levels dropped suddenly with increasing the dose to reach its lowest value in case of LD_{50} dose (2.2 ± 0.3 g/L). Data also showed a significant increase after administration of 0.1 LD_{50} daily for one, two and three months.

The concentrations of cholesterol in serum of the tested birds which administered 0.1, 0,25, 0.50 ,and 1.0 LD_{50} showed significant decrease after 24 hours, the levels were correlated negatively with the used doses. However, cholesterol content did not affect by the administration of 0.1 LD_{50} daily for one, two and three months.

Levels of triglycerides showed a significant elevation after administration of 0.1 LD_{50} for 24 hours, while administration of 0.25, 0.50 and 1.0 LD_{50} did not show any significant differences. On the other hand, triglycerides levels in serum showed a general elevation after administration of 0.1 LD_{50} for three months, but the significant increase was recorded only after one month; it increased from 3.4 ± 1.2 for control to 7.3 ± 0.7 mmol/L in the treated birds.

Concentration of serum phospholipids after administration of the different doses of chlorpyrifos showed a significant decrease at 24 hours after treatment as compared to control. The lower values were recorded in 0.5 and 1.0 LD_{50} treatments. The data did not show any significant differences in serum of the tested birds in case of administration of the low daily dose 0.1 LD_{50} after 1, 2, and 3 months.

Table (2): Biochemical parameters concentration in blood serum of quail after one, two and three months of chlorpyrifos daily oral administration by 0.1 LD_{50}.

Parameters	Period					
	1 month		2 months		3 months	
	Control	Treated	Control	Treated	Control	Treated
Ammonia (µmol/L)	713^{d}±47	1225^{bc}±193	783^{cd}±61	1923^{a}±130	588^{d}±73	1608^{ab}±166
Bilirubin (µmol/L)	16.5^{a}±1.1	$14a^{b}$±2.0	17.2^{a}±1.5	17.8^{a}±2.8	15^{a}±0.7	10.8^{b}±2.2
Glucose (mmol/L)	25.1^{b}±0.2	28.5^{b}±2.5	25.1^{b}±0.9	39.5^{a}±3.6	23.4^{b}±0.9	27.2^{b}±1.3
Total Protein (g/L)	23.8^{c}±2.0	36.7^{b}±1.8	24^{c}±0.7	41^{a}±6.6	25.5^{c}±0.5	$38.8a^{b}$±8.2
Cholesterol (mmol/L)	3.6^{a}±0.3	4.3^{a}±0.4	3.8^{a}±0.2	3.7^{a}±0.6	4.2^{a}±0.5	3.5^{a}±0.6
Triglycerides (mmol/L)	3.4^{b}±1.2	7.3^{a}±0.7	3.6^{b}±0.3	5.4^{ab}±0.4	3.2^{b}±0.2	4.5^{b}±1.2
Phospholipids (mmol/L)	5564^{a}±316	4412^{a}±459	6899^{a}±911	5472^{a}±303	6893^{a}±436	7325^{a}±489
Inorganic P (mmol/L)	$3.1a^{b}$±0.2	$4.2a^{b}$±0.5	$3.6a^{b}$±0.8	3.22^{ab}±0.5	2.12^{b}±0.4	4.6^{a}±1.3
Creatinine (µmol/L)	23^{a}±2.3	24^{a}±2.6	29^{a}±0.7	34^{a}±4.3	24^{a}±0.8	29^{a}±0.7
Urea-N (mmol/L)	2.1^{a}±0.2	0.5^{b}±0.5	2.5^{a}±0.1	1.3^{ab}±0.2	2.3^{a}±0.3	2.1^{a}±0.4
Uric acid (µmol/L)	1094^{a}±60	832^{a}±61	1191^{a}±79	895^{a}±108	1161^{a}±79	780^{a}±131

Different superscripts differ significantly at p<0.05

Concerning to the levels of inorganic phosphorus in serum, the data showed no significant differences after 24 hours of administration of 0.1, 0.25 and 0.50 LD_{50}, but administration of the LD_{50} dose resulted in a significant increase as compared to the control value, the concentrations were 2.7 ± 0.5 and 4.9 ± 0.4 mmol/L for the control and 1.0 LD_{50}, respectively. Also, administration of the daily dose equal to 0.1 of LD_{50} for one and two months did not show any significant differences when compared to control birds, while the data showed a significant increase after treatment for three months.

Creatinine content has irregular trend after 24 hours of administration with the tested doses. The one tenth of LD_{50} caused insignificant change while the content increased significantly in case of 0.25 LD_{50}. In contrast, a sudden significant drop was observed in the birds treated with 0.5 and 1.0 LD_{50}. The values were 23 ± 26, 6.4 ± 0.6 and 4.8 ± 1.5 µmol/L for control, 0.5 and 1.0 LD_{50} respectively. The concentration of creatinine did not affect by the daily low dose; 0.1 LD_{50} of chlorpyrifos for one month, two months and even for three months.

Urea nitrogen levels in serum of the treated birds after 24 hours of administration of the different single doses showed either significant or insignificant decrease compared to the control, the lowest value was recorded with the administration of a dose equal to 0.50 of the LD_{50}. The levels of serum urea nitrogen showed a general decrease after administration of the low daily dose; 0.1 LD_{50} for three months, but the decrease was significant only after administration of this dose for one month as compared to the control values.

Uric acid values in serum of the treated birds after 24 hours of administration of the different single doses showed a significant decrease comparing to the control. The levels were 1191 ± 122, 557± 46, 338 ± 27, 320 ± 11 and 720 ± 79 µmol/L for the control, 0.1, 0.25, 0.5 and 1.0 LD_{50} treatments, respectively. On the other hand, levels of uric acid did not show any significant differences after administration of 0.1 LD_{50} daily for one, two and three months as compared with control birds.

DISCUSSION

Toxicity of different insecticides as well as other pollutants for different organisms has been investigated extensively. These studies indicated that it cause damage to different organs and consequently affect the blood chemistry, (Smith, 1987 and Adham *et al.*, 2002).

In the present study, the elevation in haemoglobin (Hb) with chlorpyrifos administration was in agreement with the results previously reported by Westlake *et al.* (1981). Saad *et al.* (1973) found that Tilapia zillii exhibited an increase in RBCs of their blood under the effect of asphyxia stress. They attributed this increase due to osmotic transference of water from the blood to muscles which lead to haemoconcentration noticed. This could be the cause of increase in RBCs observed in the present work. Also, Mohamed *et al.* (1990) reported that RBCs increased in goats blood after exposure to chlorpyrifos and related this elevation to haemoconcentration.

Ammonia toxicity, measured by an increase in the blood serum ammonia, was observed in both acute and subchronic effect of chlorpyrifos to the tested birds. Such effect is in agreement with that previously reported by Radhakrishnaiah *et al.* (1995) which may reflect liver dysfunction.

Concerning bilirubin content, at general data revealed that there were a significant general decrease. These results were in agreement with Soliman *et al.* (1983) and Gomes *et al.* (1999), this decline may be attributed to dysfunction in liver and spleen as response to pesticide exposure.

Glucose levels in blood serum of the birds were generally increased either significantly or insignificantly as compared to that of control. These results agreed with those previously reported by Yassin (1998) and Adham *et al.* (2002). This elevation may be attributed possibly to a decrease in the secretary activity of the pancreatic beta cells (Matsumura, 1995) and to disturbance in carbohydrate metabolism (Abdel Aziz, 2000) Hyperglycemia could be also the result of an extraadrenal effect (Durham, 1967).

The elevation of total serum protein content after administration of low doses (0.1 and 0.25 LD_{50}) for either 24 hours or as long period as three months in the present study were in agreement with the data observed by Radhakrishnaiah *et al.* (1995). This elevation may be due to tissue damage caused by pollution which consequently leads to leakage of proteins into the blood stream.

In contrast, total protein content suddenly decreased significantly with increasing the dose to 0.5 and 1.0 LD_{50} for 24 hours. Similar results were previously observed by El-Gougary *et al.* (1999), Gomes *et al.* (1999) and Ibrahim *et al.* (2006). They suggested that pesticides and other pollutants could stimulate proteolysis by activating protease enzyme, consequently, proteolysis enhanced breakdown dominates over synthesis. Also, decline in serum protein content could be attributed also to excessive loss through nephrosis and malfunctioning of liver. However, Folmer *et al.* (1993) and Adham *et al.* (2001) concluded also that hypoproteinemia as well as hyperproteinemia may be observed in fish as a stress response.

In the current investigation, there were no effect of chlorpyrifos on both serum cholesterol and phospholipids levels after administration of the low dose (0.1 LD_{50}) daily for one, two and three months. These results agreed with the data previously reported by Enan *et al.* (1982) and Webster (1954).

However, cholesterol and phospholipids levels in serum were found to be decreased following all tested single doses. This decrease of these lipid forms may attributed to the use of these lipids as source of needed energy to mediate the effect of stress (Lee *et al.*, 1983).

Also, this study demonstrates that the level of triglycerides in serum of treated birds has not affected significantly following treatment with all tested doses and regimen except at the lowest tested single dose (0.1 LD_{50}) and shortest period of daily treatment (1 month) which showed a significant increase than in control birds. This elevation was in agreement with the data obtained by Abdel Aziz (2000) and Adham *et al.* (2002). This increase could be attributed to the faster replacement of triglycerides through the transformation of phospholipids and cholesterol into triglycerides as the first stage in metabolizing lipids into energy (Hoar, 1983).

Significant increase in serum inorganic phosphorus of the treated birds at the highest single administrated dose (LD_{50} value) and after accumulation dose of 0.1 value of the LD_{50} given daily for three months have been demonstrated. This elevation could be attributed to the breakdown of chlorpyrifos into inorganic phosphorus.

The significant decline in serum creatinine levels after administration of the higher single doses (0.5 and 0.1 LD_{50}) may be attributed to liver dysfunction since it could not be able to form creatinine from creatine. On contrast, the significant elevation observed in case of administration the single dose; 0.25 LD_{50} was in agreement with Abdel Aziz (2000) and it may be due to kidney failure (Batuman *et al.*, 1981).

Regarding uric acid content in serum of the tested birds, the significant decrement observed after all the tested single doses may be due to disturbance in protein metabolism. However, exposure of the tested birds to the oral daily dose (0.1 LD_{50}) of chlorpyrifos for three months showed that both serum creatinine and uric acid contents did not change significantly. These results agreed partially with those previously reported by Yassin (1998).

Results of the current study also lead to general decrease in the serum urea concentrations of the treated birds. These results could be attributed to liver dysfunction as respons to pesticide administration. Similar results were previously reported by El-Sebae *et al.* (1981) who concluded that in the pathological livers there was a mass action effect producing a feed back in the ornithine cycle and resulting in a type of inhibition of urea genesis.

In summary, the results of this work indicate that chlorpyrifos administration at the tested toxic doses and regimes induce significant alterations in some biochemical parameters of quail's serum. This reflects the harmful effects of this insecticide on kidney and liver functions as well as protein and lipid metabolisms.

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تأثير سمية مبيد الكلوربيروفوس على بعض الدلائل الكيموحيوية فى مصل طيور السمان

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أجريت هذه الدراسة لتقييم تأثير تعرض طيور السمان لمبيد الكلوربيروفوس على بعض الدلائل الكيموحيوية فى المصل. أوضحت النتائج أن إعطاء جرعة واحدة من الجرعات تحت المميتة بتركيزات ٠,١ ، ٠,٢٥ ، ٠,٥ ، ١ من قيمة الجرعة المميتة لنصف العشيرة (LD_{50}) عن طريق الفم وقياس الدلائل الكيموحيوية فى المصل بعد ٢٤ ساعة من تعاطى الجرعة أدى إلى إرتفاع محتوى المصل من الأمونيا والجلوكوز كما إرتفع تركيز الهيموجلوبين فى الدم بينما إنخفض تركيز كل من البيليروبين والكوليستيرول والدهون الفوسفورية ونيتروجين اليوريا وحامض البوليك. كما بينت النتائج أيضاً أن تركيز الفوسفور العضوى يزداد عند التعرض للجرعات العالية (1 LD_{50}) ويزداد تركيز الجليسريدات الثلاثية عند التعرض للجرعات المنخفضة (0.1 LD_{50}) أما محتوى المصل من البروتين الكلى والكرياتينين فلم يكن له إتجاه محدد فى التغير مع تركيزات المبيد المستخدمة.

ولقد أوضحت النتائج أن إعطاء جرعة يومية مقدارها ٠,١ من قيمة الجرعة المميتة لنصف العشيرة (0.1 LD_{50}) لمدة ثلاثة شهور وقياس الدلائل الكيموحيوية فى المصل بعد شهر وشهرين وفى نهاية مدة التجربة أدى إلى زيادة تركيز كل من الأمونيا والبروتين الكلى والجليسريدات الثلاثية والهيموجلوبين بينما يقل محتوى المصل من البيليروبين وذلك طول فترة التجربة، كما إتضح أيضاً أن تركيز الكوليستيرول والدهون الفوسفورية والكرياتينين وحامض البوليك لم يتغير معنوياً، كما وأن الجلوكوز والفوسفور غير العضوى ونيتروجين اليوريا تتغير مع الزمن بطريقة غير منتظمة. ويمكن إرجاع هذه التغيرات فى تركيب المصل والدم إلى حدوث خلل فى وظائف الكبد والكلى وضرر للأنسجة وأيضاً حدوث تنشيط أو تثبيط لبعض الإنزيمات المنظمة لعمل هذه الأعضاء تحت تأثير المبيد المستعمل.