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Toxic effect of Chloropyrpheos, Deltamethrinand Dimethoate on biochemical parameters in male rabbits.

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ABSTRACT

Dimethoate, deltamethrin and chloropyrfios are three of widely used organophosphorus insecticides in agriculture. The irrational use of Dimethoata, deltamethrin and chlyropyrfios in Libya play a crucial role in the occurrence of many diseases affecting plants, animals and the environment. The present work was conducted to investigate the alterations in biochemical parameters in male rabbits after orally administration a single dose of dimethoate (DM) by gavage at a dose of 43.2 mg/kg B.W/day (1/50 of DM) lethal dose, deltamethrin by gavages at a dose of 1.28 mg/kg B.W/day (1/50 of DM) lethal dose and chloropyrfios by gavages at a dose of 33.3 mg/kg B.W/day (1/50 of CPF) lethal dosefor 12-week.Twenty Male Rabbits weighting(1.891□27.6 Kg), were divided into four groups with 5 animals in each, first group served as control animals, they received 5 ml of corn oil, while animals in second group received Dimethoate, animals in third group received of deltamethrin and animals in fourth group received of chloropyrfios.Results obtained showed that all pesticide significantly ($P < 0.05$) induced Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (AlP) activities were increased ($P < 0.05$). All pesticide significantly ($P < 0.05$) increased the levels of plasmaglucose, urea , creatinine and total bilirubin. Conclusion, A pervasively-used dimethoate, deltamethrin and chloropyrfios helps in minimizing the damages caused by pests, but its exposure could be harmful for animal as well as human population. The present investigation provides an insight of dimethoate, deltamethrin and chloropyrfiosinduced toxicity on biochemical parameters.

الثاثير السام للكلوروبيرفيس والدلتا ميثرين والدايمثويت علي المقاييس البيوكيميائيه في ذكور الأرانب

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الكلمات المفتاحية:
الديميثوات
الديلتامثرينا
لكلوروبيرفيوس
المقاييس البيوكيميائية

الملخص

تعتبر الديميثوات والديلتامثرين والكلوروبيرفيوس من المبيدات الحشرية الفسفورية العضوية المستخدمة على نطاق واسع في الزراعة. إن الاستخدام غير الرشيد للدايميثواتا والدلتاميثرين والكليروبيرفيوس في ليبيا يلعب دوراً حاسماً في حدوث العديد من الأمراض التي تصيب النباتات والحيوانات والبيئة. تم إجراء العمل الحالي لدراسة التغيرات في المعايير البيوكيميائية في ذكور الأرانب بعد إعطاء جرعة واحدة من الديميثوات (DM) عن طريق الأنبوب بجرعة 43.2 ملغم/كغم من وزن الجسم/اليوم (1/50 من DM) جرعة مميتة، دلتامثرين. بواسطة الأقميات بجرعة 1.28 ملغم/كغم من وزن الجسم/اليوم (1/50 من DM) والجرعة المميتة بالكلوروبيرفيوس بجرعة 33.3 ملغم/كغم من وزن الجسم/اليوم (1/50 من CPF) جرعة مميتة لمدة 12 أسبوعًا. تم تقسيم عشرين أرنباً ذكراً بوزن (1.891 كغم) إلى أربع مجموعات، كل منها 5 حيوانات، المجموعة الأولى كحيوانات سيطرة، أعطيت 5 مل من زيت الذرة، بينما أعطيت حيوانات المجموعة الثانية الديميثوات، وأعطت حيوانات المجموعة الثالثة تم إعطاء الدلتامثرين وحيوانات المجموعة الرابعة للكلوروبرفيوس. أظهرت

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النتائج التي تم الحصول عليها أن جميع المبيدات الحشرية تسببت بشكل معنوي ($P < 0.05$) في زيادة نشاط ناقلة الأمين الأسبارتات (AST)، ناقلة الأمين الألانين (ALT) والفوسفاتيز القلوي ($P < 0.05$) (AlP). أدت جميع المبيدات إلى زيادة معنوية ($P < 0.05$) في مستويات بلازما الجلوكوز واليوريا والكرياتينين والبيليروبين الكلي. الاستنتاج: إن مواد الدايمثوات والدلتاميثرين والكلوروبيرفيوس شائعة الاستخدام تساعد في تقليل الأضرار التي تسببها الآفات، ولكن التعرض لها قد يكون ضارًا بالحيوانات والبشر أيضًا. يوفر البحث الحالي نظرة ثاقبة للديميثوات والديلتامثرين والسمية الناجمة عن الكلوروبيرفيوس على المعلمات البيوكيميائية.

Introduction

Bug sprays are by plan harmful. They must be poisonous to viably slaughter target species of creepy crawlies. Tragically, they too have off-target harmful impacts that can hurt other species, counting humans[1].

Observing of such kind of chemicals is critical and essential to diminish the conceivable harmful impacts upon creatures as well as open health[2]. Organophosphates, being acetylcholinesterase inhibitors, are not as it were utilized as bug sprays on assortments of cereal crops but moreover as chemical fighting specialists. In creating nations, counting Asia-Pacific locale, roughly 3 million cases of intense passings and serious cases of poisonings (0.3 million) due to organophosphate pesticides have been detailed [3]. Chlorpyrifos (CPS), also known as Chlorpyrifos ethyl, is an organophosphate pesticide that has been used on crops, animals, and buildings, and in other settings, to kill several pests, including insects and worms. It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme [4]. Chlorpyrifos was patented in 1966 by Dow Chemical Company [5].Chlorpyrifos is considered moderately hazardous to humans (Class II) by the World Health Organization based on acute toxicity information dating to 1999 [6]. Exposure surpassing recommended levels has been linked to neurological effects, persistent developmental disorders, and autoimmune disorders. Exposure during pregnancy may harm the mental development of children [7].Several studies addressed the toxic effect of dimethoate on the functions of several mammalian organs including liver and kidney. Dimethoate was reported to alter the level of the marker parameters related to the liver and kidneys in rats and mice [8]. Significant increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (ɣ-GT) as well as the decrease in the levels of cholinesterase, bilirubin, total protein and albumin in the serum were the major diagnostic symptoms of liver diseases in animals and human [9]. The increase in the uric acid and creatinine in the serum are the major symptoms of glomerular filtration damage [10]. Deltamethrin (DTM) is an important pyrethroid pesticide with broad-spectrum insecticidal activity, so it has been widely used in agriculture and aquaculture for pest and disease control [11]. With the extensive use of DTM, the substance is likely to flow into rivers and lakes through surface runoff and domestic wastewater, threatening the health of aquatic organisms, including fish [12]. Several studies have confirmed the existence of DTM in aqueous environments, with recorded values ranging from 0.73 ng/L to 24 μg/L in different localities [13]. Deltamethrin is rapidly absorbed through the intestinal epithelium and distributed into all fat-rich tissues. More than 70% of it may be 3 excreted through urine and feces in the first 24 hours after exposure [14 In past thinks about, it has been appeared that the metabolites delivered amid detoxification and biotransformation can actuate oxidative push as well as physiological and biochemical changes [15] within the cytoplasm and extracellular liquids, such as blood [16]. Observing the changes of the blood biochemical parameters can be a useful tool to analyze harmful impacts in target organs and to decide the physiological status in fowls uncovered to pesticides [17]. Changes in biochemical parameters [18], and hematologic components [19], regenerative clutters [20],neurologic al clutters [21], histopathological changes [18], and diminished survival rate of chicken and embryos [18], are detailed in feathered creatures uncovered to different pyrethroid pesticides.

Materials and methods

In this study chloropyrfios, deltamethrein and dimethoate were used. Dimethoate (purity 400g/L) was purchased from B &W agrochemichals (China), Deltamethrin were purchased as a commercial product (Butox) 50 mg/ml and chloropyrfois was purchased from public market for pesticides in Al-Bayda city. Mature male New Zealand White rabbits age of 12-week and initial weight of (1892± 50.79 Kg) were used. Animals were individually housed in cages and weighed weekly throughout 3 month experimental period.Feed and water were provided ad libtum.The first group was used as control, while, groups 2, 3 and 4 were treated with chloropyrfios 33.3 mg/kg B.W/day (1/50 of chlorpyrifos lethal dose [22]. Deltamethrin by gavage at a dose of 1.28 mg/kg B.W/day (1/50 of deltamethrin) lethal dose [23]. Dimethoate (DM) by gavage at a dose of 43.2 mg/kg B.W/day (1/50 of DM) lethal dose [24] for 12 successive weeks. The other part of the parted blood samples were placed immediately on ice. Plasma was obtained by centrifugation of samples at 860 xg for 20 C until used for analyses. Stored plasma samples°min, and was stored at -20 were analyzed for Plasma concentrations of cholesterol and triglycerides (TG) were determined according to the methods of [25]. High-density lipoprotein (HDL) was determined according to the methods of [26]. Low density lipoprotein (LDL) was determined by the calculation (cholesterol-(TG/5+HDL). The activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of [27]. Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma according to the method of [28]. Liver thiobarbituric acid-reactive substances (TBARS) were measured by the method of[29].Statistical analysis was carried out in Minitab software (version 17). Statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the data and appropriate $P < 0.05$ consider significant.

Results

All the rabbits (control and treated) were observed daily after every dosing for 3-5 hrs for clinical symptoms like salivation, hyperactivity, irritability, faecal pellet conditions, diarrhea, weakness, coarse tremor, paralysis of limb, convulsions, wounds, vocalization, aggressive behaviour, ataxia, increased sensitivity to external stimuli, unsteady gait, falling of hair, stress (fur erection and exophthalmia) and changes in non-sexual behaviours (such as cleaning of face, excessive self grooming, climbing in cages).All the rabbits (control and treated) were observed daily after every dosing for 3-5 hrs for clinical symptoms like salivation, hyperactivity, irritability, faecal pellet conditions, diarrhea, weakness, coarse tremor, paralysis of limb, convulsions, wounds, vocalization, aggressive behaviour, ataxia, increased sensitivity to external stimuli, unsteady gait, falling of hair, stress (fur erection and exophthalmia) and changes in non-sexual behaviours (such as cleaning of face, excessive self grooming, climbing in cages).All treatments were for 12 weeks. The present study demonstrated that oral treatment of rabbits with chlorpyrifos (33.3mg/kg body weight), deltamethrin(1.28mg/kg body weight)and dimethoate (43.2mg/kg body weight)-treated rabbits, **(**Figure 1**)** showed hair loss, nausea, dizziness, dry mouth, indigestion, abdominal pain, vertigo, vomiting, constipation, drowsiness, and headache especially in the last four weeks of the experiment. Observation of animals dimethoate-fed rabbits showed varying degrees of clinical signs few minutes after dose. The signs included disorientation,

drowsiness, uncoordinated movements, mild tremor, Redness around eyes, blindness, diarrhea and hair loss especially in the fifth and sixth weeks of the experiment(Figure 1) whereas control animals did not display such change. The liver of dissected rabbits also showed scars of depression in response to dimethoate administration (Figure 1), in comparison with normal control animals group showed normal appearance.

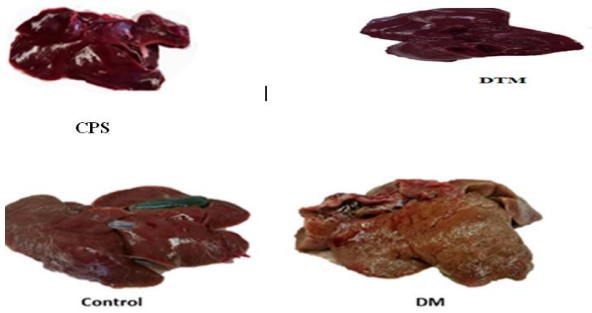

Figure 1. Morphological effect of CPS, DTM and DM after 12 weeks on liver.

Tables 1 and Figures 2 to 9 represented the mean values of activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AlP) and bilirubin in plasma of male rabbits treated with chloropyrfios (CPF), deltamethrin (DTM) anddimethoate (DM).Treatment with chloropyrfios (CPF), deltamethrin (DTM) and dimethoate (DM).caused a significant (p<0.05) increase in plasma bilirubin AST, ALT, AlP activities glucose, urea and creatinine. While, decrease plasma levels of TP, albumin.

Table 1.The activities of plasma enzymes of male rabbits treated with chloropyrfios (CPS), deltamethrin (DTM) and dimethoate (DM).

	Experimental groups			
Enzyme	**CON**	**CPS**	**DTM**	**DM**
AST (U/L)	36.1 ± 0.779^{b}	46.36 ± 2.098^{a}	47.11 ±2.316^{a}	46.89±0.700^{a}
ALT (U/L)	28.09 ±1.958^{c}	24.83 ±0.621^{c}	37.8 ± 2.902^{b}	84.6 ± 1.214^{a}
ALP (IU/L)	50.68 ±2.182^{b}	53.18 ± 2.763^{b}	51.4 ± 2.473^{b}	135.8 ± 1.81^{a}
Bilirubin (mg/dL)	1.50 ± 0.029^{b}	1.6600.033^{a}	1.56 ±0.050^{ab}	1.650± 0.010^{a}
albumin (mg/dl)	3.678± 0.091^{a}	3.448± 0.098^{a}	3.511± 0.101^{a}	3.568± 0.055^{a}
Glucose (mg/dl)	115.2± 0.409^{b}	120.03 ± 1.316^{a}	120.34±1.174^{a}	118.5 ± 0.37^{ab}
Urea (mg/dl)	37.43±0.522^{b}	42.13± 1.793^{a}	43.45± 1.568^{a}	39.85±0.377^{ab}
Creatinine (g/dl)	0.758±0.032^{b}	1.181± 0.074^{a}	1.198 ±0.079^{a}	0.738±0.013^{b}

Values are expressed as means ± SE; n = 5 for each treatment group.Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, p<0.05.

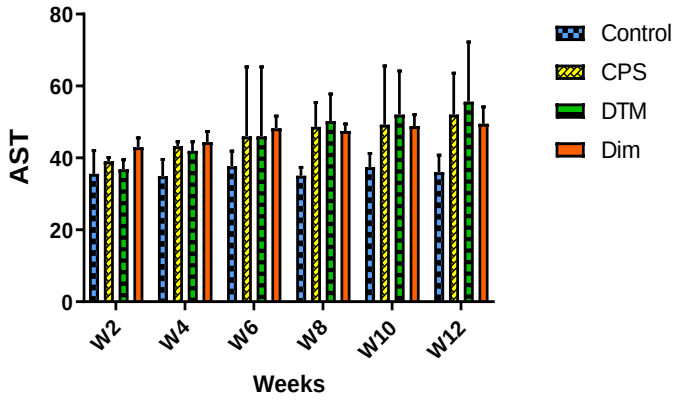

Figure 2. Changes in aspartate transaminase (AST) duringtreatment of male rabbits with chloropyrfios (CPF), deltamethrin (DTM) anddimethoate (DM).

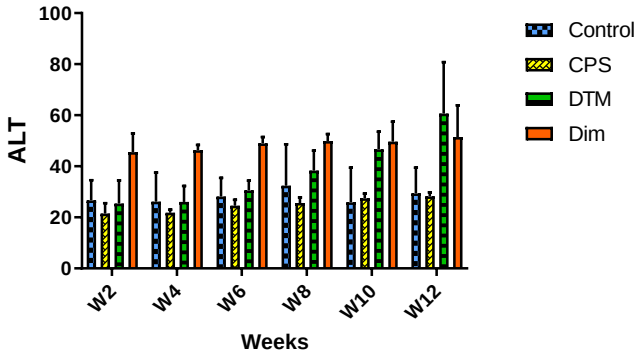

Figure 3. Changes in alanine transaminase (ALT) during treatment of male rabbits with chloropyrfios (CPF), deltamethrin (DTM) anddimethoate(DM).

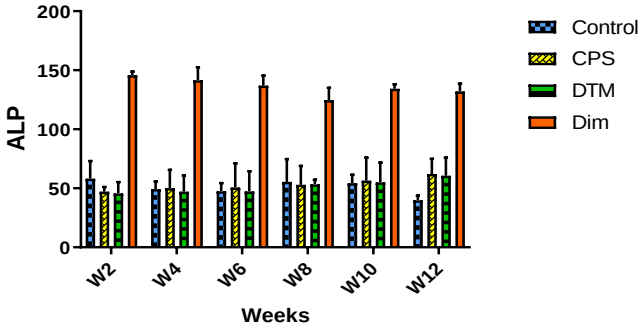

Figure 4. Changes in alkaline phosphatase (ALP) during treatment of male rabbits with chloropyrfios (CPF), deltamethrin (DTM) anddimethoate (DM).

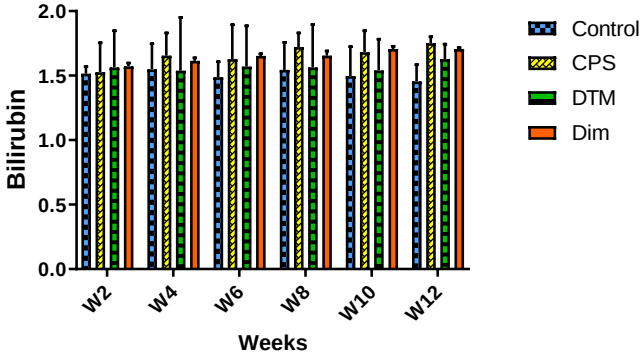

Figure 5. Changes in T-biliriubin during treatment of male rabbits with chloropyrfios (CPF), deltamethrin (DTM) and dimethoate (DM).

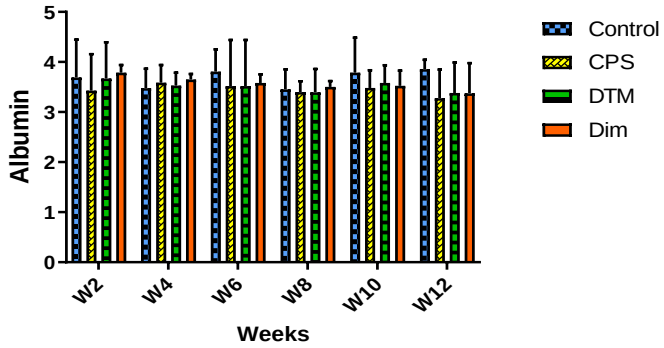

Figure 6. Changes in values of plasma albumin during treatment of male rabbits with **chloropyrfios** (CPF), deltamethrin (DTM) anddimethoate (DM).

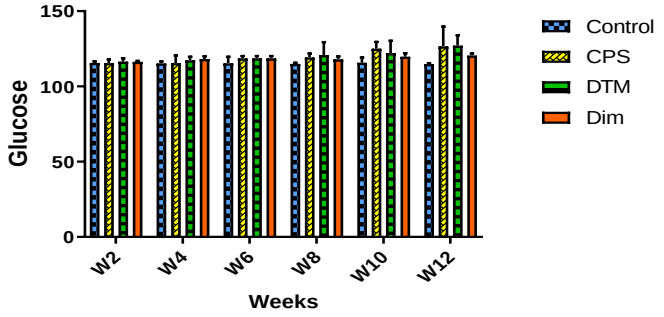

Figure 7. Changes in values of plasma glucose during treatment of male rabbits with chloropyrfios (CPF), deltamethrin (DTM) anddimethoate (DM).

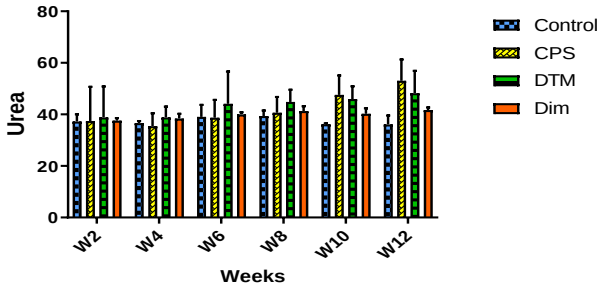

Figure 8. Changes in urea during treatment of male rabbits with chloropyrfios (CPF), deltamethrin (DTM) anddimethoate (DM).

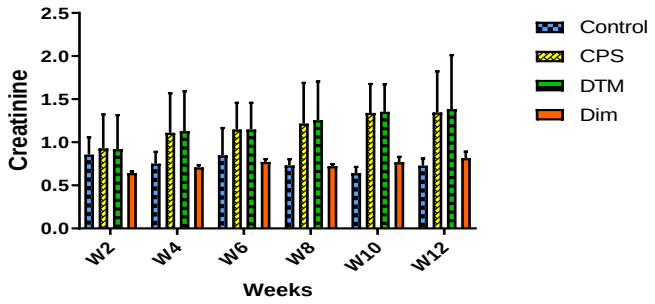

Figure 9. Changes in creatinine during treatment of male rabbits with chloropyrfios (CPF), deltamethrin (DTM) and dimethoate (DM).

DISCUSSION

The livers of dimethoate-treated rabbits showed scars of depressions also in the last two weeks of the experiment which may be due to distortion in the liver cells. Dimethoate is known to induce morphological changes in the liver [30].Data presented in this study showed that the mean levels of serum ALT, AST, ALP and bilirubin in the chlorpyrifos-treated rabbits were significantly higher than those in the controls. Such elevation of liver enzymes as a result ofchlorpyrifos administration was documented by other authors [31]. Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as chlorpyrifos poisoning [32]. Serum ALT, AST and bilirubin are considered to be among the most sensitive markers employed in the diagnosis of hepatotoxicity [33]. In the present study oral administration of chlorpyrifos caused gradual increase in bilirubin level throughout the experiment. Such increase was reported previously by [34-35] in chlorpyrifos in toxicated rats. The change in serum bilirubin which is accepted as indicator of liver function may provide further evidence on hepatotoxicity induced by the organophosphorus insecticide chlorpyrifos [36].

The influence of chlorpyrifos on kidney function was assessed through the measurement of urea and creatinine. Urea concentration was generally increased throughout the whole experiment, and this increment becomes significant during the last four weeks of the experiment compared to the control. For creatinine this increase was also observed along the whole experiment with significant increments in 4th and 5th weeks of the experiment. Such findings are in agreement with that reported in other studies [37,7,4]. A creatinine level raised out of proportion to the urea may indicate a pre-renal problem [38]. Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function[39]. Increase in serum urea observed in the present study may be due to impairment in its synthesis as a result of impaired hepatic function, disturbance in protein metabolism and decrease in its filtration rate in the kidney. The decrease in protein profile observed in the present study may support this explanation. Creatinine is break-down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys [40]. Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired for any reason, for example in case of chlorpyrifos poisoning, the creatinine level in the blood will rise due to poor clearance by the kidneys. A rise in blood creatinine level is observed with damage to functioning nephrons and impaired renal function [41,42]. The current results showed significant increase in plasma ALT and AST activities (Table 2). The increase in activity of these enzymes in plasma is indicative for liver damage and thus causes alteration in liver function [43] and [44]. In addition, [45] found that cell damage exhibited good correlation with the enzyme leakage. Hence, cellular damage caused by toxic substances is This decrease in plasma protein could be attributed to changes in protein and free amino acids metabolism and their synthesis in liver. Also, the observed decreases in plasma proteins could be attributed to the damaging effect of deltamethrin on liver c ells as confirmed by increasing in the activities of ALT and AST. The observed decrease in the ALP activity in the plasma of quails fed diets contaminated with deltamethrin may indicate severe damage to the liver [46]. [47] found that the decreased ALP activity could be related to a defect in the biosynthesis of this enzyme in the liver of rats exposed to deltamethrin. Furthermore, hemolysis and lipid peroxidation of erythrocytes may be due to the decreased ALP activity in the blood of birds treated with deltamethrin.[48] reported that hemolysis of erythrocytes leads to the release of electrolytes such as magnesium and zinc, which in turn may have an inhibitory effect on the ALP activity. The results of this study revealed a significant increase in the serum AST activity by the acute and chronic deltamethrin exposure, which agreed with the findings of [49]. Serum AST is present in all tissues of the body, it is not an organ specific enzyme and consequently may be utilized to detect destruction in a wide variety of tissues including liver [50]. [51] attributed the elevated serum AST activity of eel (Anguella anguella) to liver damage. However other organs as kidney may have also been damaged. [49] came to the same conclusion, which agreed with the liver, and kidney findings of this study. The increased AST activity reflects an increase in protein breakdown in various tissues [52]. The increased activity of AST was observed in the plasma of rats exposed to deltamethrin [43] and the plasma of Cyprinus carpio exposed to chlorpyrifos [53]. Nevertheless, no significant changes were observed in the ALT activity in the blood of quails fed diets contaminated with

deltamethrin when compared with the control group. Although some other studies have reported the increased serum ALT activity following prolonged pesticide exposure [54,55], the findings of this study suggest that the plasma ALT may not be a good clinical marker of prolonged sub-lethal deltamethrin treatment. At the end of the experiment, the ALP activity in the plasma of the individuals treated with deltamethrin decreased significantly compared with the control group ($p < 0.05$). The observed decrease in the ALP activity in the plasma of quails fed diets contaminated with deltamethrin may indicate severe damage to the liver [46]. [47] found that the decreased ALP activity could be related to a defect in the biosynthesis of this enzyme in the liver of rats exposed to deltamethrin. [56] reported that the reduction in plasma protein, particularly albumin, in animals treated with pesticides could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. Birds fed diets with 0.25 mg/kg deltamethrin also exhibited raised glucose levels, but this group was not significantly different from the control group. The plasma glucose level was significantly higher in birds treated with 0.5 mg/kg deltamethrin than that in the control group ($p < 0.05$). Hyperglycemia, observed in quails fed with deltamethrin-contaminated diets, indicates impaired glucose metabolism and glycogen degradation in liver [53].

Conclusion

In conclusion,the results of the present study convincingly demonstrated that chloropyrfios (CPS), deltamethrin (DTM) anddimethoate (DM) exposure resulted in varying degree of biochemical parameters in plasma of rabbits .Pesticides have adverse effects on the health of rabbits . According to our findings, we may conclude that chloropyrfios (CPS), deltamethrin (DTM) and dimethoate (DM) causes biochemical changes in alterations in some biochemical characteristics, and enzymatic activities.

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