Change in acetylcholine activity and some blood parameters in adult sheep dipped in deltamethrin

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Abstract
The aim of this was to elucidate the effects of deltamethrin dipping on acetylcholine activity in serum and some blood parameters of sheep. The study was conducted on forty adult sheep dipped in deltamethrin in concentration of 1:1000L of water. Venous blood samples were collected from each animal before and after dipping. There was significant inhibition in the acetylcholine esterase in all subjected animals after dipping. Mean values of hematological investigations showed a significant rise in the number of total leucocytes (leukocytosis) with significant decline in hemoglobin concentration and Red cell indices in all dipped animals after dipping in deltamethrin when compared with mean values obtained from the same animals before dipping. Conclusion of our results revealed that poisoning with deltamethrin affects blood parameters through the inhibition of acetylcholine esterase.

Keywords: Acetylcholine, Deltamethrin, Acetylcholine esterase activity, Sheep, Blood parameters

Introduction
Organophosphorus compound and carbamate pesticides are broadly used in public health (1), veterinary practice, and in agriculture (2). They pose main environmental pollution complications and health threats to people and animals (3,4). These pesticides inhibit cholinesterase (ChE) activity in the nervous tissues and neuromuscular junctions, causing an accumulation of acetylcholine at the nerve endings which afterward produces signs of toxicosis characterized by nicotinic, muscarinic, and central nervous system effects (5,6). The Cholinesterase activities in blood, tissue and brain can be easily measured (7,8) and their investigations are useful in determining the exposure status of cholinesterase inhibitors and detecting the toxicity of these compounds (8-10). Acetylcholinesterase activity inhibition have been widely studied red blood cells, plasma and tissues in workers (11,12), experimental animals (13,14), fish (9), birds (15,16) and in farm animals (8,17) after exposure to organophosphate and carbamate pesticides but rarely studied in serum (18) specially in our country and adverse effect on blood parameters. One of the basic mechanisms of the harmfulness of the pesticides seems to be lipid peroxidation; as a consequence, these composites can lead to some blood cells disorders (19). So this study was designed to elucidate the effects of deltamethrin on acetylcholine and some blood parameters of sheep dipped in deltamethrin.

Material and methods
Sample collection
This study was conducted on forty adult sheep dipped in pesticides. Venous blood samples were collected from each animal; one before dipping (used as control, and the second sample collected after dipping, then each sample was divided into two parts; first part used for complete blood pictures study and the remainder was centrifuged, after clotting, at 2000-3000 rpm for 20 minutes, and the separated serum was immediately frozen at -20°C for later estimation of Ach esterase activity.
Methods
Blood parameters red blood cells (RBC) count, white blood cells (WBC) count, differential leucocyte count, hematocrit, hemoglobin concentration, red cell indices estimated by automated machine (Ruby- automated hematology analyzer) (20). Acetyl choline esterase assayed quantitatively in vitro by using the enzyme linked immunosorbent assay (ELISA) kit (RelAssay Diagnostics) based on the principle of biotin double antibody sandwich technology.

Dipping
Animals were stopped giving water for 12 hours. One hour prior to dipping they were allowed one hour for taking water. Then All subjected animals were dipped in a basin, 0.9*1.5*3 m in dimension, containing deltamethrin 25 g/L (DELTDIP®) in concentration1:1000L for 30 seconds.

Statistical analysis
Data obtained were analyzed using the Excel program version 10 package. The significance of invariant differences was assessed by student’s t-tests and all values were two-sided. A P<0.01 was considered statistically significant.

Results
There was significant decrease in the acetylcholine esterase activity in all subjected animals after dipping as compared to pre-dipping period (Figure 1). The mean values were 5.74 and 1.24±0.31 nmol/Ml before and after dipping respectively.

Figure 1: Mean values± SD of Acetylcholine esterase activities in dipped sheep before -and- after dipping in deltamethrin.

Mean values of hematological investigations showed a significant rise in the number of total leucocytes with significant decline in hemoglobin concentration in all animals after dipping in pesticides when compared with mean values obtained from the same animals before dipping (table1). Regarding the differential leucocyte count, our result showed significant increase in the granular (neutrophils and eosinophils) and some granular leucocytes (lymphocytes and monocytes). Mean values of Red cell indices; Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC), also showed significant decrease. There was a decrease in the mean value of red blood cells (RBCs) count and hematocrit (Hct) but was statistically no significant.

Table 1: Mean values ± SD of blood parameters in dipped sheep before -and- after dipping in deltamethrin

|        | WBC 10⁹/ m³ | LYM % | MON % | NEU % | EOS % | RBC m/m³ | MCV (fl) | Hct (%) | MCH (pg) | MCHC (g/dL) | HB (g/dL) |
|--------|-------------|-------|-------|-------|-------|-----------|----------|---------|----------|-------------|-----------|
| Before | 13.40       | 44.06 | 13.1  | 23.15 | 0.26  | 4.0       | 32.49    | 27.86   | 80.99    | 10.3        |
| dipping| ±1.30       | ±1.06 | ±1.25 | ±0.7  | ±0.03 | ±0.37     | ±0.8     | ±0.83   | ±0.82    | ±1.23       | ±0.38     |
| After  | 19.7*       | 62.6* | 16.6* | 26.6  | 0.61* | 3.77      | 28.4*    | 28.13   | 16.36*   | 75.8*       | 8.7*      |
| dipping| ±1.8        | ±1.86 | ±1.63 | ±0.93 | ±0.07 | ±0.36     | ±28.46   | ±0.53   | ±1.04    | ±0.71       | ±0.34     |

-P<0.01 statistically significant.
Discussion

Organophosphorus compounds (OP) is progressively increasing in Iraq, most likely because of the comprehensive availability of pesticides as a result of wide-ranging use in agriculture, veterinary and also because of over the counter sale of these items. Current results showed a significant decrease in acetylcholine activities in the sera of sheep after dipping in pesticides when compared with the values obtained after dipping. This conclusion confirms the effect of these compounds on acetylcholine activity and put forward that estimation of serum choline esterase is useful in conformation of OP poisoning. The reported results are similar to the recorded results in other studies (21). When acetylcholine esterase is inactivated, it leads accumulation of acetylcholine throughout the nervous system and causes hyperstimulation of muscarinic and nicotinic receptors (22).

Accumulation of free radicals resulted from acetylcholine esterase activity inhibition leads to lipid peroxidation (23). Free radicals contribute in toxicity of pesticides which may persuade oxidative stress resulting in releasing of free radicals and modification in antioxidants or oxygen free radical scavenging enzyme system (24). In a study, Handekari et al (19), reported that cells constantly suffer from oxidative stress in spite of over activity of antioxidant defense mechanism indicated by increase in erythrocyte. Increase in antioxidant enzymes levels may be essential to purify increased concentration of lipid peroxidation yields that are oxidative stress products because of organophosphorus poisoning (23).

The significant increase in leucocytes observed after dipping in pesticides implies the activation of the defense mechanism and immunity. The same observation was reported by Hundekari (19) that the number of white blood cell count was increased significantly in all cases of OP poisoning. They also detected increased in total white blood cells with mild decrease in neutrophils count as compared to control values. In acute OP toxicity, overproduction of reactive O2 species immediately results in major lipid peroxidation. The liability of erythrocytes and leucocytes especially lymphocytes to oxidative stress due to exposure to the pesticide is a function of overall equilibrium between degree of oxidative stress and antioxidant protection proficiency. Thus the antioxidant attack aptitude of exposed subjects may alter directly or indirectly by OP compounds and therefore affect their liability to oxidative stress of exposed subjects and consequently affect their vulnerability to oxidative stress (24).

There is significant decline in the mean values of hemoglobin concentration of animals after dipping compared to the mean values before dipping, possibly due to the decline in formation of hemoglobin or may be due to binding of organophosphorus insecticides on iron, followed by a lack of fusion of iron with hemoglobin leading to low MCV, i.e. decreased size of erythrocytes (microcytosis).

Conclusion

From the above results it was concluded that organophosphorus compounds cause a significant decline in AChE activity and the severity of poisoning aids in predicting OP poisoning and effects on blood parameters. These effects may be through oxidative damage caused by elevating lipid peroxide levels.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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التغير في نشاط الأستيت كولين وبعض معايير الدم في الأغنام الخاضعة في مبيد دلتاميثريين

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الخلاصة

هدف الدراسة هو توضيح تأثير مركب الفسفور العضوي المستخدم في تغطيس الأغنام على نشاط الأستيت كولين وبعض معايير الدم في الأغنام الخاضعة. تم جمع نماذج الدودة من كل حيوان قبل وبعد تغطيس الأغنام، ثم تم قياس نشاط الأستيت كولين باستخدام شرائح ميكروليبيريبلاستيك في جميع الحيوانات خاضعة بعد الغطس. كما أظهرت التحليلات المطلوبة المرتبطة الموادية ارتفاعًا ملحوظًا في عدد الكريات الدموية مع ارتفاع الدهون في ركز الكريات الدموية للحيوانات بعد الغطس. كما اخذت النتائج التي توصلنا إليها أن التamespace دلتاميثريين يؤثر على معايير الدم من خلال تأثيرات استمترنر التي تؤثر على نشاط الأستيت كولين.

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