

Toxicological and neurobehavioral effects of chlorpyrifos and deltamethrin insecticides in mice

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Abstract

The aim of the present study was to determine the acute toxicity of chlorpyrifos and deltamethrin in mice separately and to study their toxic and neurobehavioral effects. Median Lethal Doses (LD₅₀) of chlorpyrifos and deltamethrin were determined depending on up and down method. The oral LD₅₀ of chlorpyrifos was 193.05 mg/kg and of deltamethrin was 15.71 mg/kg in mice. The oral administration of chlorpyrifos 155 mg/kg and deltamethrin 12.56 mg/kg represent 80% of LD₅₀ resulted in acute signs of poisoning that manifested by dyspnea, salivation and lacrimation at 100%, piloerection, straub tail, tremors, convulsions and death at 70% for chlorpyrifos and 60% for deltamethrin and writhing reflex at 20% for chlorpyrifos. Oral administration of chlorpyrifos 310 mg/kg and deltamethrin 24 mg/kg increased severity of toxicosis signs as a percentage of piloerection, straub tail, tremors, seizures and death 100%. As well as decrease the onset of tremors, convulsions and death, writhing reflex which appears at 20% for chlorpyrifos and 10% for deltamethrin. After three hours of chlorpyrifos and deltamethrin oral administration at doses represent 20% and 10% of LD₅₀ there are significantly hypoactivation in open-field activity, significantly increased in the duration of negative geotaxis performance, significantly decreased in head poking and swimming scores compared to control group. In conclusion we found that deltamethrin was more toxic than chlorpyrifos this is based on the LD₅₀ value. However, the signs of toxicosis and neurobehavioral effects produced by both toxicants were not differential and could not be associated with the toxic level.

Keywords: Avian influenza, RRT-PCR, Histopathology, Rapid test, Broilers

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التأثيرات السمية والسلوكية العصبية لمبيد الحشرات الكلوربايرفوس والدلتامثرين في الفئران

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الخلاصة

هدف الدراسة الحالية كان الكشف عن السمية الحادة المحدثة بالكلوربايرفوس والدلتامثرين كل على حدة في الفئران ودراسة تأثيراتهما السمية والسلوكية العصبية. تم تحديد الجرعة المميّنة الوسطية (الجم-٥٠) لكل من الكلوربايرفوس والدلتامثرين في الفئران كل على حدة بالاعتماد على طريقة الصعود والنزول، وكانت الجرعة-٥٠ للكلوربايرفوس ١٩٣,٠٥ ملغم/كغم والدلتامثرين ١٥,٧١ ملغم/كغم عن طريق التجريب بالفم في الفئران. أدى إعطاء الكلوربايرفوس بجرعة ١٥٥ ملغم/كغم والدلتامثرين بجرعة ١٢,٥٦ ملغم/كغم للفئران كل على حدة والتي تمثل ٨٠٪ من الجرعة-٥٠ إلى إظهار علامات التسمم كصعوبة التنفس والالعب والتدمع بنسبة ١٠٠٪ وانتصاب الشعر والذيل والرجفة والاختلاجات العصبية والموت بنسبة ٧٠٪ مع الكلوربايرفوس وبنسبة ٦٠٪ مع الدلتامثرين مع ظهور منعكس التمطي بنسبة ٢٠٪ مع الكلوربايرفوس فقط، وسبب الكلوربايرفوس بجرعة ٣١٠ ملغم/كغم والدلتامثرين بجرعة ٢٤ ملغم/كغم عن طريق الفم ارتفاع نسب ظهور علامات التسمم كانتصاب الشعر والذيل والرجفة والاختلاجات العصبية والموت الى ١٠٠٪ مع الكلوربايرفوس و٨٠٪ مع الدلتامثرين بالإضافة لانخفاض اوقات بدء علامات التسمم وبدء الرجفة والاختلاجات وحدوث الموت مع ظهور منعكس التمطي بنسبة

٢٠٪ في الفئران المعاملة بالكلوربايرفوس وبنسبة ١٠٪ في الفئران المعاملة بالدلتامثرين. بعد ٣ ساعات من إعطاء الكلوربايرفوس والدلتامثرين بجرع تمثل ٢٠٪ و ١٠٪ من الجسم-٥٠ أحدث المبيدين انخفاضا معنويا في النشاط الحركي داخل الميدان المفتوح مع زيادة معنوية في وقت إكمال اختبار الانتحاء الأرضي السالب وانخفاضا معنويا في عدد مرات إدخال الراس في الثقوب ومراتب السباحة مقارنة بمجموعة السيطرة. استنتجت دراستنا أن الدلتامثرين أكثر سمية من الكلوربايرفوس بالرجوع إلى الجرعة المميتة الوسطية لكل مبيد. من جهة أخرى كانت علامات التسمم والتأثيرات السلوكية العصبية الظاهرة على الفئران لكلا المبيدين غير تفريقيه وغير متعلقة بمستوى السمية.

Introduction

Insecticides known as chemicals manufactured and used to control insects like lice, ticks and used in veterinary medicine to control and eradicate mites and mange (1,2). Chlorpyrifos a broad-spectrum insecticide belongs to organophosphorus compounds. It is used in public health, agricultural crops and in livestock to control different pests (3,4), chlorpyrifos eliminates and eradicates insects by direct exposure or in contact with treated surfaces. Chlorpyrifos classified as a moderate toxic compound within organophosphorus compounds in mice and rats and LD₅₀ ranged between 50-500 mg/kg body weight (5), as well as have moderate toxicity in sheep and Guinea pigs (3). Toxic effects of chlorpyrifos by affecting normal function of the insect nervous system (6) by irreversible inhibition of acetyl cholinesterase enzyme (AchE) that is responsible for the breakdown of acetylcholine (Ach) (7-10). After exposure to the toxic doses, signs of poisoning appear within a few minutes or after few hours this depending on the degree of absorption, distribution, metabolism of insecticide inside the body and its excretion (11). Chlorpyrifos poisoning shows the cardinal signs of organophosphorus compounds that is represented by muscarinic signs, which appears early that includes: salivation, lacrimation, sweating, colic with diarrhea, dyspnea with profuse nasal discharges, urination, defecation, miosis and bradycardia (12,13), followed by nicotinic signs that includes: muscle fasciculation, muscle twitching, abnormal gait, in coordination and tremors (14), eventually nervous system effects which includes hyperactivity, anxiety, tremors followed by convulsions, depression and finally death as a result of respiratory failure (15,16). Deltamethrin is a broad-spectrum insecticide classified as type II synthetic pyrethroid (17), pyrethroids are esters analogs of natural pyrethrins (extract of the flower *chrysanthemum cinerariaefolium*) (18), pyrethroids are used to control and eradicate many pests (19). Deltamethrin has central nervous action and it has cyanide group that linked with sodium channels gaits located in the cellular membrane which lead to produce prolonged inhibition of sodium channels gaits and continuous sodium ions flow so it lowers the action potential threshold and prolonged depolarization resulting in continuous stimulation and hyper activation of the nerve cells (20,21). As deltamethrin

classified type II synthetic pyrethroid it shows choreoathetosis with salivation (CS-syndrome) (22). In rodents the appearance of the signs beginning with pawing, burrowing, followed by salivation, dyspnea, in coordination, choreoathetosis followed by seizures and in the late stages there is occurrence of rolling convulsions which are the specific sign of deltamethrin poisoning in rodents and eventually death due to the respiratory failure (23).

Due to the wide disturbing, handling and using of organophosphorus compounds and pyrethroids in veterinary medicine and in agriculture, we select chlorpyrifos and deltamethrin that currently used in the markets of Mosul, we aimed in this study was to compare and to study the toxicological and neurobehavioral effects of chlorpyrifos and deltamethrin in mice.

Material and methods

Animals

We used 120 white males and females' mice, their ages between 60-90 days, and their body weight between 20-34 g, which housed in the animal house under laboratory conditions 12/12 hours light - dark cycle with water and food ad libitum.

Doses preparation

A commercial insecticide emulsified solution of chlorpyrifos 48% concentration (Tarkim, Turkey) and deltamethrin 2.5% concentration (Indogulf, India), they diluted with distilled water freshly prepared every day before using. The volume of administration was 10 ml/kg B.Wt. given orally.

Determination of the oral median lethal dose LD₅₀ of chlorpyrifos in mice by up and down method

Six male and female mice were used and their body weight ranged between 24-34 g, firstly chlorpyrifos dosed orally at 300 mg/kg depending on previous studies and trails, the result was read death X or life O after 24 hour, and the amount of dose increased or decreased was constant 50 mg/kg and repeat dosing up or down for 3mice after changing the result death to life and versa and calculate chlorpyrifos LD₅₀ depending upon the diagram and equation of Dixon (24) $LD_{50} = Xf + Kd$, in which Xf: last

dose, K: diagram value, d: the value of dose increases or decreases.

Determination of the oral median lethal dose LD₅₀ of deltamethrin in mice by up and down method

Eight male and female mice were used, their body weight ranged between 26-30 g, firstly deltamethrin orally dosed at 30mg/kg depending on previous studies and trails, the result was read death X or life O after 24 hours, and the amount of dose increased or decreased was constant 5 mg/kg and repeat dosing up or down for 3mice after changing result death to life and versa and calculate deltamethrin LD₅₀ depending upon the diagram and equation of Dixon (24) that mentioned previously.

Acute toxic effects of high toxic doses of chlorpyrifos and deltamethrin in mice

Forty male and female mice were used, their body weight ranged between 22-32 g, groups were orally dosed, directly after oral dosing the mice were observed carefully and acute toxic signs of chlorpyrifos and deltamethrin were recorded during 4 hours, these groups as follows:

Acute toxic effects of 80% of LD₅₀ of chlorpyrifos and deltamethrin: Twenty mice divided into two groups (10 mice for each group). The first group was orally dosed with chlorpyrifos at 155 mg/kg B.Wt. While second group was orally dosed with deltamethrin at 12.56 mg/kg B.Wt.

Acute toxic effects of double doses from A of chlorpyrifos and deltamethrin: Twenty mice divided in to two groups (10 mice for each group). The first group was orally dosed with chlorpyrifos at 310 mg/kg B.Wt. While second group was orally dosed with deltamethrin at 24 mg/kg B.Wt.

Neurobehavioral effects of low doses of chlorpyrifos and deltamethrin in mice

Sixty male and female mice were used, their body weight ranged between 20-29 g, groups were orally dosed and divided as follows:

Neurobehavioral effects of 20% of LD₅₀ of chlorpyrifos and deltamethrin: Thirty mice divided in to 3 groups (10 mice for each group). The first group (control) was orally dosed with distilled water. The second group was orally dosed with chlorpyrifos at 38.6 mg/kg B.Wt. The third group was orally dosed with deltamethrin 3.14 mg/kg B.Wt.

Neurobehavioral effects of 10% of LD₅₀ of chlorpyrifos and deltamethrin: thirty mice divided in to 3 groups (10 mice for each group). The first group (control) was orally dosed with distilled water. The second group orally dosed with chlorpyrifos at 19.3 mg/kg B.Wt. The third group orally dosed with deltamethrin 1.57 mg/kg B.Wt.

After 3 hours of oral administration of chlorpyrifos and deltamethrin, all mice subjected to neurobehavioral tests.

Open- field activity test: This test measures the locomotors behavior, mice were subjected to the open-field activity test by using 35×35×25 cm box, the arena of the box was divided into 25 equal squares, and each intended mouse to measure placed in the center of arena and account the number of squares crossed in its four legs, number of rearing, fecal boluses and urination pools within 3 minutes (25).

Negative geotaxis: This test measures neuro-motor coordination, vestibular function and the associated integrative motor response to space orientation, this behavior was measured on slope rough wooden surface at angle 45° by placing each mouse head down position on the center of an inclined surface and the mouse was timed for completing 180° turn, and maximum time allowed was 60 second (26).

Head pocking: The test used to measure locomotivity curiosity and movement of treated mouse by using a plastic circular surface it's diameter 30 cm with low edges and 10 cm height, the circular surface contain 8 holes 2 cm each, the mouse placed on the surface and account the head pocking in holes for 3 minutes (27).

Swimming test: This test measures treated mouse response to stressful condition which requires coordination of nerve high centers and neuromuscular responses, this test performed by dropping a mouse in a glass aquarium with the water height 30 cm and 29-30 C° temperature for 5-10 seconds and watching the mouse carefully and scores recorded (28).

Statistical analysis

The parametric results were statistically analyzed by using one-way analysis of variance (ANOVA) test version 16.0, then subjected to least significant difference test depending on statistical analysis program SPSS (29). Were the non parametric results statistically analyzed by using fisher exact test and mann whitny U test, and the level of significance was P<0.05 (30).

Results

Determination of the oral LD₅₀ of chlorpyrifos in mice by up and down method

The oral LD₅₀ of chlorpyrifos was 193.05 mg/kg body weight after giving different doses with appearance of toxic signs represented by excitation, salivation, lacrimation, defecation, urination, dyspnea, tremors, muscle fasciculation, convulsions, straub tail, piloerection, also writhing reflex in some treatments and eventually death at high toxic doses (Table 1).

Determination of the oral median lethal dose LD₅₀ of deltamethrin in mice by up and down method

The oral LD₅₀ of deltamethrin was 15.71 mg/kg body weight with appearance of the toxic signs which represented by excitation, choreoathetosis with salivation, lacrimation, defecation, urination, dyspnea, tremors, muscle fasciculation, rolling convulsions, straub tail, piloerection, also writhing reflex in some treatments and eventually death at high toxic dose (Table 2).

Table 1: Chlorpyrifos LD₅₀ and toxic signs in mice

Measurements	Result
Chlorpyrifos LD ₅₀	193.05 mg/kg orally
Doses range	300-150=150 mg/kg
First dose	300 mg/kg
Last dose	150 mg/kg
Up and down dose	50 mg/kg
No. of mice	6 (XXOXXO)
Onset of toxic signs	5-23 minutes
Toxic signs	Excitation, salivation, dyspnea, tremors, muscle fasciculation, convulsions, death

O: mouse still life during 24 hours, X: mouse dead during 24 hours.

Table 2: Deltamethrin LD₅₀ and toxic signs in mice

Measurements	Result
Deltamethrin LD ₅₀	15.71 mg/kg, orally
Doses range	30-10=20 mg/kg
First dose	30 mg/kg
Last dose	15 mg/kg
Up and down dose	5 mg/kg
No. of mice	8 (XXXXOXXO)
Onset of toxic signs	5-18 minutes
Toxic signs	Excitation, choreoathetosis, tremors, salivation, rolling convulsions, death

O: mouse still life during 24 hours, X: mouse dead during 24 hours.

Acute toxic effects of high toxic doses of chlorpyrifos and deltamethrin in mice

Acute toxic effects of 80% of LD₅₀ of chlorpyrifos and deltamethrin

Oral administration of chlorpyrifos at dose 155mg/kg B.Wt. and deltamethrin at dose 12.56 mg/kg B.Wt, in mice led to appearance the signs of poisoning that includes: dyspnea, salivation, lacrimation, which appears in both treatments at 100%, also piloerection, straub tail, tremors, convulsions and death appears in chlorpyrifos treatment at a percentage that more than in the deltamethrin treatment, as well as there is significant increase in the onset of signs

time, onset of convulsions time and in death time with chlorpyrifos in comparison with deltamethrin, and increase in the onset of tremors time with chlorpyrifos in comparison with deltamethrin, and writhing reflex appears in some chlorpyrifos treatments at 20% (Table 3).

Acute toxic effects of double doses from A of chlorpyrifos and deltamethrin

The oral administration of chlorpyrifos at dose 310 mg/kg B.Wt. and deltamethrin at dose 24 mg/kg B.Wt., in mice led to appearance of the signs of poisoning including: dyspnea, salivation, lacrimation, that appears in both treatments at 100%, also piloerection, straub tail, tremors, convulsions and death appears in chlorpyrifos treatment at percentage more than deltamethrin treatment, also there is increase in the onset of signs time and the onset of tremors time of chlorpyrifos in comparison with deltamethrin, as well as there is significant increase in the onset of convulsions time and in death time with chlorpyrifos in comparison with deltamethrin. Writhing reflex appears in some chlorpyrifos and deltamethrin treatments at 20% and 10%, respectively (Table 4). The double doses of both insecticides that used in this experiment showed an increase in the toxic sign's incidence, intensity and severity.

Table 3: Acute toxic signs induced by chlorpyrifos 155mg/kg and deltamethrin 12.56 mg/kg PO in mice

Signs of toxicosis %	Chlorpyrifos	Deltamethrin
Dyspnea	100	100
Salivation	100	100
Lacrimation	100	100
Defecation	100	70
Urination	100	80
Piloerection	70	60
Straub tail	70	60
Tremors	70	60
Convulsions	70	60
Writhing reflex	20	Zero
Death	70	60
Onset of signs time	24.63±2.16*	19.5±0.98
Onset of tremors	83.1±1.99	67.75±4.74
Onset of convulsions	91.1±1.98*	85.13±4.43
Onset of death	149.6±6.6*	126.88±5.69

Values are mean ± SE for 10 mice / group, * The value significantly different from the group treated with Deltamethrin at P<0.05.

Neurobehavioral effects of low doses of chlorpyrifos and deltamethrin in mice

This experiment showed that neurobehavioral effects of group A were more pronounced than group B, as well as neurobehavioral effects of chlorpyrifos in group B was more significant than in deltamethrin in the same group.

Neurobehavioral effects of 20% of LD₅₀ of chlorpyrifos and deltamethrin

The oral administration of chlorpyrifos at dose 38.6 mg/kg B.Wt and deltamethrin at dose 3.14 mg/kg B.Wt, led to behavioral changes after 3 hours of the treatment which is represented by a significant decrease in the open field activity (squares crossed and rearing) in comparison with the control group, a significant increase in the time spent for the negative geotaxis performance in comparison with the control group, and a significant decrease of head pocking, swimming scores in comparison with the control group, and a significant increase in urination and defecation in comparison with the control group (Table 5).

Table 4: Acute toxic signs induced by chlorpyrifos 310 mg/kg and deltamethrin 24 mg/kg orally, in mice

Signs of toxicosis%	Chlorpyrifos	Deltamethrin
Dyspnea	100	100
Salivation	100	100
Lacrimation	100	100
Defecation	100	80
Urination	100	80
Piloerection	100	80
Straub tail	100	80
Tremors	100	80
Convulsions	100	80
Writhing reflex	20	10
Death	100	80
Onset signs time	19.1±1.43	13. ±0.99
Onset of tremor time	64±4	55.3±5.6
Onset of convulsions	76±5*	64.17±2.03
Onset of death	108.3±2.26*	75.2±4.75

Values are (mean ± SE) for 10 mice /group, * The value significantly different from the group treated with deltamethrin at P<0.05.

Neurobehavioral effects of 10% of (LD₅₀) of chlorpyrifos and deltamethrin

The oral administration of chlorpyrifos at dose 19.3 mg/kg and deltamethrin at dose 1.57 mg/kg B.Wt., led to behavioral changes after 3 hours of the treatment which is represented by a significant decrease in open-field activity (squares crossed and rearing) in comparison with the control group and a significant increase in the time spent for the negative geotaxis performance in comparison with the control group, and a significant decrease in the head pocking, swimming scores in comparison with the control group, and increase in urination and defecation in comparison with the control group (Table 6).

Discussion

The oral LD₅₀ of chlorpyrifos in mice was 193.05 mg/kg B.Wt, in the previous study the LD₅₀ of chlorpyrifos in mice was 436.05 mg/kg body weight (31) this attributed to the differences in the manufacturing processes, preparations composition, variation of origin, as well as the solvents, the intermediate vehicles, the emulsifiers which added to be solved in water and the concentration of the active ingredient in the formula as well as season and laboratory temperature (32), It is worth to mention that chlorpyrifos from moderately toxic organophosphorus compounds their LD₅₀ ranging between 50-500 mg/kg, chlorpyrifos moderately toxic to mice and rats as well as sheep, guinea pigs (3,5), but it is highly toxic in birds and poultry (7), non-vertebral aquatic organisms and fish (33), rabbits were resistant to chlorpyrifos toxicity due to their own levels of paraoxygenase enzyme that responsible for the analysis of chlorpyrifos Oxon (metabolic product of chlorpyrifos).

Chlorpyrifos and chlorpyrifos Oxon increase the production of the chlorpyrifos Oxon from the metabolism of chlorpyrifos in the liver metabolic pathways by CYP450 and lead to the increase in acetylcholinesterase inhibition percentage, poisoning signs and toxic effects will be more harmful (34).

Table 5: Neurobehavioral effects of 20% of LD₅₀ of chlorpyrifos 38.6 mg/kg and deltamethrin 3.14 mg/kg PO in mice

Variables	Treatment		
	Control group	Distilled water	
Squares crossed / 3 min		70 ± 4.75	26 ± 4.6*
Rearing / 3 min		18.76 ± 1.78	9.9 ± 0.6*
Negative geotaxis (seconds)		4.5 ± 0.37	12 ± 1.01*
Head pocking 3 / min		25.1 ± 1.38	6 ± 0.9*
Fecal boluses / 3 min		0.33 ± 0.16	1.5 ± 0.22*
Urination pools / 3 min		0.11 ± 0.11	0.8 ± 0.13*
Swimming scores		4 ± 0	3.2 ± 0.13*

Values are (mean ± SE) for 10 mice /group, * The value significantly different from the control group at P< 0.05.

Table 6: Neurobehavioral effects of 10% of LD₅₀ of Chlorpyrifos 19.3 mg/kg and Deltamethrin 1.57 mg/kg PO in mice

Variables	Treatment		
	Control group Distilled water	Chlorpyrifos	Deltamethrin
Squares crossed / 3 min	69.4 ± 4.1	53.1 ± 3.17*	58.5 ± 3.54*
Rearing / 3 min	20.2 ± 0.64	12.2 ± 1.17*	14.1 ± 1.36*
Negative geotaxis (seconds)	3.9 ± 0.27	9.9 ± 0.5 ^{*,a}	8.9 ± 0.7*
Head pocking 3 / min	29.1 ± 1.4	14.2 ± 1.39 ^{*,a}	18.8 ± 1.28*
Fecal boluses / 3 min	0.4 ± 0.16	1.2 ± 0.13*	0.7 ± 0.13
Urination pools / 3 min	0.2 ± 0.13	0.6 ± 0.16*	0.13 ± 0.15
Swimming scores	4 ± 0	3.7 ± 0.15*	3.8 ± 0.13*

Values are mean ± SE for 10 mice /group, * The value significantly different from the control group at P< 0.05, ^a the value significantly different from deltamethrin treatment at P< 0.05.

The oral LD₅₀ of deltamethrin in mice was 15.71 mg/kg B.Wt, it is known that deltamethrin LD₅₀ ranged between 19-34 mg/kg body weight in deltamethrin preparations that contain vegetable oil or polyethylene glycol as a vehicle (35), the additives in deltamethrin preparations affects the LD₅₀ value by affecting the absorption rate and lipophilic one will rapidly be absorbed (3).

Deltamethrin metabolized in liver through the breakdown of the ester bond, the only pathway for deltamethrin metabolism in the body, deltamethrin has the toxic effects without its own nontoxic metabolites (23,36). The 80% of LD₅₀ of chlorpyrifos and deltamethrin were selected and orally dosed equal to 155, 12.56 mg/kg B.Wt., respectively, to observe and record the toxic signs that induced in these doses.

The mechanism of toxic action of chlorpyrifos after exposure and systemically absorbed was the inhibition of AchE in the neural tissue causing accumulation of Ach in the synaptic cleft so there is stimulation in the muscarinic and nicotinic receptors (13,37) chlorpyrifos oral administration with 155 mg/kg B.Wt., which led to the appearance of poisoning signs that begin with muscarinic signs that manifested by dyspnea, salivation, lacrimation, defecation and urination, followed by the nicotinic signs and CNS effects that manifested by piloerection, straub tail, tremors followed by convulsions, also writhing reflex appeared in some treated mice and the signs ended with death due to the respiratory failure, these are the ideal signs of organophosphorus compounds (38).

Deltamethrin oral administration with 12.56 mg/kg B.Wt., led to the appearance of specific poisoning signs of type II synthetic pyrethroids which manifested by (choreoathetosis with salivation syndrome) (22) signs begins with chewing, salivation, pawing, piloerection, straub tail, tremors, rolling convulsions (specific signs of deltamethrin poisoning in rodents), eventually death because of respiratory failure (23), poisoning signs which appeared linked to the mechanisms of deltamethrin toxic action principally on the modulation of sodium channels gait which present in the cellular membrane of the neuron

which prolong the inhibition of sodium channels gait and continuous sodium ions flow so it prolonged depolarization and resulting in the continuous stimulation and hyper activation of the nerve cells (20) also deltamethrin affects chloride ion channels as anti GABA receptors which prevents chloride ions flows (39), so this explains the occurrence of tremors, as well as deltamethrin affects Ca Mg ATPase resulting in the increase of intracellular calcium level which led to increase the stimulation of neurotransmitters release like Ach (22).

Doubling of the toxic doses will lead to increase the severity of the toxic signs and increase in their incidence and harm, as well as will increase in the death percentage (40,41), this observed when we give double doses of chlorpyrifos 310 mg/kg B.Wt., and deltamethrin 24 mg/kg B.Wt., Which led to increase in the tremors, convulsions and death percentages, this differences in the toxic effects for insecticides depending on the differences in the toxicokinetic, toxicodynamic that responsible for the differences in the extent of toxicity (42,43). Many studies depend on the neurobehavioral tests, which reveal the hidden negative effects that do not appear clearly when exposed to sub acute and chronic exposure of many insecticides that they do not appear overt poisoning signs and reflect their toxic effects on the nervous and muscular systems and their balance together (44).

In the current study many behavioral tests have been measured that reveal different types of behavioral functions such as open-field activity test which lowers the motor activity due to inhibition in the central nervous system (25), and negative geotaxis test, which increases the duration of negative geotaxis performance due to the defect in the vestibular geotaxis (26), and head pocking test that reveals the negative effects on the brain and it's cognitive function (27), In addition to the swimming test that decreases due to the defect in the coordination between the central nervous system and the muscular system and defects of the muscular system that response to the nervous system stimulation (28).

The results of this study in mice that treated with chlorpyrifos and deltamethrin at low toxic doses are significant deceased in open-field activity (squares crossed and rearing), head pocking, swimming scores and significant increase in the duration of negative geotaxis performance in comparison with the control group, These behavioral changes are identical in the cases of organophosphorus compounds and pyrethroids poisoning in subacute and chronic exposure (45-47).

The neurobehavioral effects of the organophosphorus compounds at low toxic doses do not principally depend on the AchE inhibition, but depend on the effects of other neurotransmitters like dopamine, serotonin and GABA (48,49,50), as well as neurobehavioral effects of pyrethroids which appears due to weakening of the neuromuscular response in low toxic doses (47,51).

Conclusion

In conclusion, the acute toxic manifestations and severity of toxicity in chlorpyrifos and deltamethrin are similar to the signs of toxicosis and neurobehavioral effects that found with chicks (46,52) and other animals when exposed to different toxicants with different mechanism of action.

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