Neurobehavioral evaluation of quail treated with omega-3 and chlorpyrifos

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**Abstract**

The exposure of animals to pesticides through contamination of feed or water is a problem that needs solutions. This study considered. In the first axis, different chlorpyrifos LD50 Concentration was used, equaling 5.6, 11.2, and 16.7 mg/kg, respectively. In the second axis, the most hazardous dose was chosen. Here, the quails were separated into four groups of 5 animals. The first group acted as a control, whereas the second and third received Omega-3 250 mg/kg orally daily for one month. The third group was given Chlorpyrifos at 16.7 mg/kg once (after a month of Omega-3 treatment), whereas the fourth group received only 16.7 mg/kg once orally. Chlorpyrifos had an LD50 of 55.83 mg/kg. Locomotor activity and nervous behavior were inhibited at 11.2 and 16.7 mg/kg. It was linked to an increase in oxidative stress with a decrease in the total antioxidant status. In contrast, the Omega-3 and Chlorpyrifos 16.7 mg/kg group showed lower neuro-behavioral toxicity and maintained the antioxidant status in the quail's body. In contrast, the group treated with chlorpyrifos alone showed toxic effects. Neuro-behavioral and decreased total antioxidants with a high level of malondialdehyde with the appearance of histopathological effects in brain tissue and affected neurons, whereas the brain tissue of the Omega-3 for a month with chlorpyrifos group did not show pathological Changes in comparison to the chlorpyrifos alone. This study indicates that antioxidant status can indicate pesticide toxicity and may prevent its toxicity.

**Keywords**: Brain, Antioxidant, Pesticide, Animals

**Introduction**

Pesticides are chemicals used in agriculture to help food production by preventing and controlling harmful insects (1). Pesticide use contributes to significant environmental damage and health concerns (2,3). Chlorpyrifos (CPF) is an example of this class of insecticide. Because of its mechanism of action against insects, CPF is a broad-spectrum insecticide that is commonly used in agriculture (4). Using pesticides produces free radical, which causes oxidative damage in various cells (5). Organisms' bodies fight themselves against oxidative stress caused by free radicals by using an internal and extrinsic system within the cells (6,7). Recently, researchers have looked into the effect of natural dietary antioxidants in preventing oxidative stress damage (8). Omega-3 is an essential fatty acid in practically all biological systems and is utilized as a food supplement among antioxidants (1,2). It also boosts antioxidant levels, helping to protect lipid membranes from oxidative stress and its damaging effects (9). In recent animal research, omega-3 fatty acids have been revealed to play an essential role in increasing acetylcholine, serotonin, and dopamine levels in the brain (10). Research has shown that these fatty acids are essential in enhancing mental abilities and improving mood, are necessary for insertion, behavior, and memory, and are related to the functional structure of the cell membrane by interfering with phospholipids, which form neuron membranes important in the biological integrity of the cell (11). The interaction of omega-3 is reflected in the structure of the brain and nervous system of the animal (12). The open field test observed a significant increase in the behavioral performance of rats treated with 500 mg/kg (13). Recent
research indicates a substantial impact of essential fatty acids on behavioral patterns and their association with oxidative damage (14).

The current study sought to determine how omega-3 supplementation over four weeks protects quail body levels of nervous behavior and motor function and its effect on oxidative stress after chlorpyrifos poisoning.

Materials and methods

Animals

Used an adult quail (Coturnix japonica) 2-3 months old and weighed 300 to 400 grams. The animals were bred in the College of Vet-Med animal house at the University of Mosul, where they were given water and food during the research time, under acceptable laboratory settings and considering the adaptation period in birds.

Ethical approval

All honest permissions have been obtained from the College of Veterinary Medicine, University of Mosul. Humane dealing with animals was approved. The form number was UM-VET-2022.06.

Pharmaceuticals and chemicals

Using omega-3 from an American company and chlorpyrifos 48% from Lorcepan company, Dow AgroSciences, France. Total Antioxidant Capacity Kit from Labor Diagnostika Nord LDN Germany. Distilled Water was used to dilute chlorpyrifos. Initially, the LD50 of chlorpyrifos was measured using the Down method (15) in quails when administered orally.

The first axis was designed to determine the dose-response for different concentrations of chlorpyrifos. Animals are grouped into four groups, each with five animals. The first group was given distilled water as a control, and the second, third, and fourth groups were given chlorpyrifos at concentrations of 10%, 20%, and 30% of the LD50, which equates to 5.6, 11.2, and 16.7 mg/kg. The respective doses were administered orally once daily (single dose). G1 was control group. G2 was 10%of LD50 chlorpyrifos. G3 was 20%of LD50 chlorpyrifos. G4 was 30%of LD50 chlorpyrifos

After completing the previous axis, the most appropriate toxic dose is selected, which is 16.7 mg/kg, to apply this dose in the second axis. The second axis of the quail divides into four groups of five animals. The first group was the control group, whereas the second and third were given Omega-3 250 mg/kg orally daily for a month. The third group received 16.7 mg/kg chlorpyrifos once dose orally (after a month of Omega-3 delivery), and the fourth group received chlorpyrifos only 16.7 mg/kg once orally. G1 was control group. G2 was treated with omega-3 250 mg/kg for one month. G3 was treated with omega-3 250 mg/kg, then Chlorpyrifos 16.7 mg/kg single dose. G4 was treated with chlorpyrifos 16.7 mg/kg.

Following the end of the two-axis treatment period, we perform neuro-behavioral assessments in the open field after 24 chlorpyrifos treatments. We calculate the number of cut squares, jumps out of the box, defeation times, and the tensile immobility test. After the neuro-behavioral examinations, the birds’ jugular veins were cut to collect blood. The samples were then put in sterile tubes and left at room temperature. For 15 minutes, allow blood to clot before being transferred to a centrifuge at 3000 rpm for 15 minutes to separate serum. Finally, the brain is removed and placed in 10% neutral formalin-containing containers until tissue slicing is conducted.

Acetylcholine esterase activity

Measurement by using modified-electrometric method (16). 3 mL of distilled water, 0.2 plasma, and 3 mL of phosphate buffer solution are mixed together, and the pH of the mixture is measured before adding 0.1 acetylcholine iodide solution as a base material. The mixture is transferred to a 37°C water bath and incubated for 30 minutes before measuring the second pH. Then, using the equation: control without inhibition - enzyme activity with inhibitor / enzyme activity in control, the difference between the two values is computed to determine the degree of inhibition.

Total antioxidant capacity (TAC)

is measured using an ELISA kit and a specific measurement kit. All instructions are included in the measuring kit.

Malondialdehyde (MDA) measurement

The method of Aust and Buege is used (17). MDA's interaction with thiobarbituric acid (TBA) was a complicated component of MDA-TBA2, and it was strongly absorbed at 352 nm. The ultimate malondialdehyde concentration of MDA is calculated using the equation below. Micromol/L = (Sample absorbance - absorption efficiency/MDA)*10⁶.

Statistical investigation

The results were shown, including the mean and standard error; the parameter data was analyzed using SPSS software using analysis of variance (ANOVA), and the LSD test was utilized. The Mann-Whitney test was used to assess the non-parametric data in the previous program; the probability level is less than 0.05.

Results

The Dixon up and down method calculated the median lethal dose (Table 1), 55.83 mg/kg orally. Chlorpyrifos at 11.2 and 16.7 mg/kg inhibited animal activity, with a decrease in activity as measured by the number of cut squares, defeation, and leaping times and increased tonic immobility response time in comparison to the control and the second group (Table 2). The data demonstrated that
giving omega-3 supplements for one month positively affects neurotoxicity reduction. There was no substantial variance in neuro-behavioral assessments compared to control when the animals were treated with omega-3 250 mg/kg for one month and then administered chlorpyrifos at 16.7 mg/kg. The animals' sleep duration improved compared to the fourth group. The antioxidant status improved in the same group, with a lower concentration of malondialdehyde compared to the fourth group (Tables 3 and 4). Histological slices of the brain were made to clarify the apparent histopathological effects on brain tissue, and those effects in omega-3 were identical to the control group in the presence of normal histological structure in the brain tissue (Figures 1 and 2). The existence of histopathological effects in the brain tissue of animals treated with chlorpyrifos 30%. This is reflected by congestion of blood vessels, diffuse perivascular and peri-axonal edema, glial cell satellitosis around neurons, and neuronophagia (Figure 3). The apparent effects on brain tissue in the group treated with omega-3 for one month and then administered chlorpyrifos were minor and less than what was observed in the chlorpyrifos group alone (Figure 4).

Table 1: oral LD50 of chlorpyrifos in quails

<table>
<thead>
<tr>
<th>Variables LD50 after 24 hours</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD50 after 24 hr</td>
<td>55.830 mg/kg</td>
</tr>
<tr>
<td>The dosages range</td>
<td>120-30.0 mg/kg</td>
</tr>
<tr>
<td>A first dose</td>
<td>120. mg/kg</td>
</tr>
<tr>
<td>A final dose</td>
<td>30. mg/kg</td>
</tr>
<tr>
<td>Quail Number</td>
<td>(XX0XX0) %6 animals</td>
</tr>
<tr>
<td>Dose increase or decrease</td>
<td>30. mg/kg</td>
</tr>
<tr>
<td>Toxicity indicators</td>
<td>Depression, lack of movement, and other anxiety symptoms.</td>
</tr>
</tbody>
</table>

* X denotes death, and O indicates quail survival.

Table 2: The activities in the open field and the tonic immobility response in quails

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time of onset start (s)</th>
<th>Squares (n/3min)</th>
<th>Jumping (n)</th>
<th>Defecations (n)</th>
<th>Tonic immobility response time (h/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>1±1</td>
<td>19±2.5</td>
<td>5±2</td>
<td>3±1</td>
<td>48±5</td>
</tr>
<tr>
<td>G2</td>
<td>1±1</td>
<td>18±1</td>
<td>5±1</td>
<td>3±1</td>
<td>51±2</td>
</tr>
<tr>
<td>G3</td>
<td>1±1</td>
<td>15±2^A</td>
<td>2±1^A</td>
<td>2±1</td>
<td>100±14^A</td>
</tr>
<tr>
<td>G4</td>
<td>2±1</td>
<td>15±2.0^A</td>
<td>3±1.0^AB</td>
<td>2±1</td>
<td>110±2^AB</td>
</tr>
</tbody>
</table>

Each one group has five animals. Data is shown as mean and SE. (*) this signifies that the control group is different. (A) this indicates a considerable deviation from chlorpyrifos 10%. (B) mean significant difference from chlorpyrifos 20%.

Table 3: Quail open-field activity and length of tonic immobility response treated by Omega-3 and Chlorpyrifos

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time of onset start (s)</th>
<th>Squares (n/3min)</th>
<th>Jumping (n)</th>
<th>Defecations (n)</th>
<th>Tonic immobility response time (h/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>2±1</td>
<td>21±2.5</td>
<td>5±2</td>
<td>3±1</td>
<td>48±5</td>
</tr>
<tr>
<td>G2</td>
<td>2±1</td>
<td>20±1</td>
<td>4±1</td>
<td>3±1</td>
<td>50±2</td>
</tr>
<tr>
<td>G3</td>
<td>2±1</td>
<td>19±2^A</td>
<td>4±1</td>
<td>2±1</td>
<td>60±2^A</td>
</tr>
<tr>
<td>G4</td>
<td>3±1</td>
<td>15±2.0^AB</td>
<td>3±1.0^AB</td>
<td>2±1</td>
<td>110±2^AB</td>
</tr>
</tbody>
</table>

Each one group has five animals. Data is shown as mean and SE. (*) this signifies that the control group is different. (A) this indicates a considerable deviation from omega-3. (B) mean significant difference from omega-3 and chlorpyrifos.

Table 4: Cholinesterase and total antioxidant capacity and malondialdehyde levels in quails

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholinesterase inhibition level (ΔPH)</th>
<th>Total antioxidant capacity (U/ml)</th>
<th>Malondialdehyde level (Mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>1.6±0.3</td>
<td>1.105±0.02</td>
<td>0.096±0.01</td>
</tr>
<tr>
<td>G2</td>
<td>1.55±0.2</td>
<td>2.218±0.06*</td>
<td>0.109±0.1</td>
</tr>
<tr>
<td>G3</td>
<td>1±0.1</td>
<td>1.90±0.06</td>
<td>0.113±0.02</td>
</tr>
<tr>
<td>G4</td>
<td>1.8±0.1</td>
<td>0.599±0.01^AB</td>
<td>0.713±0.01^AB</td>
</tr>
</tbody>
</table>

Each one group has five animals. Data is shown as mean and SE. (*) this signifies that the control group is different. (A) this indicates a considerable deviation from omega-3 250 mg/kg. (B) mean significant difference from chlorpyrifos 16.7 mg/kg.
Discussion

Our findings show that quails LD50 equals 55.83 mg/kg, more significant than the value observed in chicks in a prior study, where the deal was 25-35 mg/kg. That result refers to quail being less vulnerable to chlorpyrifos poisoning than chicks (More resist than chicks) (18,19). Chlorpyrifos is known to inhibit cholinesterase, which increases acetylcholine and consequently increases the animal's motor activity (20). This contradicts the findings of other studies in which rats, mice, or chicks were given chlorpyrifos, which decreased locomotor activity and neurotic behavior. These findings suggested that other factors, including oxidative stress, influence neurobehavioral and motor activity.

Exacerbated oxidative stress, cellular damage in the brain, intracellular imbalance, particularly Ca2+, increased expression of signaling of Interleukins and cytokines, as well as enhanced activity/expression of protein kinases, including protein kinases, psyllium- and mitogen-activated kinases, have all been proposed to contribute to CPF neurotoxicity (19-21).

Pesticide toxicity is linked to oxidative damage produced by increased free radical production. The findings revealed that the quantities of thiobarbituric acid reactive compounds raised in the serum of treated quails were dramatically elevated with a concentration of 30%.

Omega-3 plays a significant part in the integrity of the neurological system and transmission of nerve impulses between cells and enhances specific neurotransmitters such as serotonin, dopamine (22,23). Our findings revealed that omega-3 could protect quails from low doses of chlorpyrifos, with effects on neurobehavioral, motor activity, and oxidative stress, which lines up with several studies showing omega-3's ability to improve cognitive and behavioral functions, as well as its role in increasing antioxidant status in the body (23,24).
Chlorpyrifos and other pesticides cause alterations in neurobehavior and motor activity in animals, as well as metabolic and neurotransmitter abnormalities (24,25). Because of the diverse types and breeds of birds, we recorded the LD50 chlorpyrifos in quail, which differs from what was recorded in the rest of the birds. The challenge of supplying omega-3 to chlorpyrifos in a low dose resulted in a favorable outcome in measuring neurobehavior, which could be owing to omega-3 causing an increase in serotonin concentration and releasing dopamine storage in presynaptic arteries and, therefore, changing behavior. Dopamine receptors are activated or modulated post-synaptically due to post-synaptic activation or modulation (25,26). However, the opposing effect of chlorpyrifos is counterbalanced by the fact that harmful low-dose organophosphorus insecticides are toxic at low concentrations and inhibit the neurotransmitters serotonin, dopamine, and GABA but not acetylcholine (26,27).

Omega-3s increase mental abilities, mood, cognition, behavior, and memory. They are linked to the cell membrane’s functional structure by interfering with phospholipids necessary for cell integrity (27-29). DHA is vital for brain development and function because it is found in high concentrations in the gray matter of the brain, and it is the chemical that encourages the membranes of neurons to fulfill their duties necessary for brain signal transmission by making their membranes more flexible (30-32). In quail, serotonin depletion is critical in heightening fear and discomfort when quails were given a 20 and 30% LD50, reflected in an increased sleep in the stress immobility response test.

According to recent research, omega-3 influences behavioral profiles and their association with oxidative stress (33,34). They boost antioxidant status in the body and have an important effect on behavioral profiles and their association with oxidative stress (35-37). The delivery of chlorpyrifos to quails resulted in a reduction in TAC, accompanied by an increase in malondialdehyde (38). This supports the theory that chlorpyrifos causes oxidative stress by generating free radicals that harm cellular components (39,40).

Conclusions

According to the findings of this study, antioxidant status can be used as a possible indicator of pesticide toxicity. Quails given omega-3 at 250 mg/kg for four weeks could protect their bodies against poisoning with modest chlorpyrifos doses.

Acknowledgment

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Conflict interest

No competing interest.

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