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Research Article

Effects of Diazinon on Biochemical and Histological of Liver Quail Birds (*Coturnix sp.*) Under Laboratory Conditions in Misurata City, Libya

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Abstract

Continuous exposure of living organisms to pesticides causes various diseases of the liver. It leads to effects on the enzymes involved in vital functions in both humans and animals. This study utilized the pesticide diazinon to investigate its oral dosing effects on certain biochemical and histological functions of the livers of local quail (*Coturnix sp*). The Cobas Integra 400 device from Roche was used to measure serum enzymes (AST, ALT and ALK). Additionally, histological sections were prepared using a rotary microtome and H&E staining was employed. The current study indicated the impact of diazinon on the biochemical measurements and histological structure of the kidneys and livers of local quail. The current study indicated an increase in both AST, ALT and ALK, with each increase being statistically significant (P<0.05). The histological examination of the livers of quail treated with diazinon revealed numerous pathological changes. Microscopic analysis of the liver indicated necrosis and degeneration in the hepatic tissue, along with a transformation in the shape of liver cells from their normal polygonal form to an enlarged and irregular shape. Additionally, there was evidence of rupture in the connective tissue that supports the liver components, along with hemorrhaging in the major blood vessels and sinusoids. Furthermore, microscopic examination revealed fibrosis in the endothelium of the central vein.

Keywords: Biochemistry; Diazinon; Enzymes; Histology; Liver; Quail

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Introduction

Pesticides are available for all types of insect pests, contributing to the production of high-quality food products that remain undamaged by pest infestations. Additionally, their use leads to increased crop yields, which helps lower market prices. This approach also prevents pest-related issues that can result in food shortages for consumers [1,2].

Initially, pesticides demonstrated good efficacy; however, improper use has led to significant harm to humans, animals, and plants. This is primarily because they can indirectly kill other living organisms, disrupting the natural biological balance and consequently impacting biodiversity. Additionally, farmers may be affected by inhaling the spray while applying pesticides to crops. Fish residing in rivers, springs, and lakes are also harmed when these chemicals seep into water sources. Furthermore, birds drinking from exposed water sources near pesticide contamination can suffer adverse effects. These pollutants can transfer to humans and animals either directly or indirectly through the food chain, leading to accumulation in various tissues [3].

The effects of pesticides on specific organs and tissues, as well as on the biochemistry of living organisms, are among the primary concerns of researchers in this field. This is particularly important given the variety of pesticides and the complex interactions between chemical substances and various environmental and industrial factors, most of which are reactive and unstable [4].

The populations of birds in agricultural lands worldwide are declining, yet the contribution of pesticides to this decline requires extensive study. Most research focuses on the effects of these agricultural chemicals (pesticides) under laboratory conditions (in vitro), determining the lethal dose for fifty percent of the test animals (LD_{50}) and testing a limited number of bird species. Most of these birds belong to the order Galliformes, which play a significant role in maintaining their ecosystems and are often raised by humans for their meat and eggs [5].

Organophosphate (OP) and carbamate pesticides are neuroactive compounds that inhibit the enzyme acetylcholinesterase. These pesticides are widely utilized as miticides and insecticides in agricultural, household, and garden settings. Notable OPs, whether currently in use or historically, include fenthion, fenamiphos, parathion, chlorpyrifos, malathion, famphur, phorate, terbufos, and diazinon. Common carbamates include carbofuran, aldicarb, and carbaryl [6].

The study on the spatial and temporal distribution of diazinon indicated that it poses a low to moderate chronic risk to organisms inhabiting sediments and a low toxic effect on aquatic invertebrates, primarily concentrated in areas of high human density at Baiyangdian lake [7].

Diazinon, an organophosphate, is often recognized as a significant cholinesterase inhibitor in cases of unintentional avian toxicity and mortality [8]. Generally, diazinon undergoes rapid degradation in natural environments; however, findings have been inconsistent, and some degradation products may possess toxicity comparable to that of the parent compound. Diazinon demonstrates high acute toxicity to a diverse array of animal species, resulting in various sublethal biochemical effects, damage to specific target organs and tissues, cytotoxic and genotoxic effects, reproductive impairment, and negative ecological consequences [9].

Diazinon toxicity has been documented across a wide range of avian species. The study investigating the effects of diazinon on the reproductive system of *Colinus virginianus* indicated a decrease in egg production, as well as a reduction in food consumption [10]. Meneely and Wyttenbach [11], studied the effects of the insecticide on the eggs of quail embryos, demonstrating a significant inhibition of acetylcholinesterase activity compared to the control sample. Additionally, the study conducted by Ismail [12], which focused on quail using the insecticides cypermethrin and diazinon, reported a decrease in the weights of the experimental birds as well as a reduction in the weights of the embryos. While there are no studies addressing the effects of diazinon on the biochemical and histopathological damage to the liver of quail, this research represents the first investigation into the effects of diazinon on the liver of quail. Therefore, the current study aimed to evaluate the impact of a sublethal dose of diazinon on the biochemical and histological aspects of the liver following oral administration under laboratory conditions.

Materials and Methods

Study Samples

Quail are among the most commonly used laboratory animals due to their small size, low maintenance costs, and rapid reproduction. Their body length does not exceed 20cm, with an average weight ranging from 150-245grams, and an average egg weight of 8 to 15grams. Quail belong to the class Aves, order Galliformes, family Phasianidae, and genus Coturnix [13]. 40 local quail (*Coturnix* sp) were used as experimental animals, with weights ranging from 200 to 250grams and ages of 40 days. The animals were housed in the animal house at the Faculty of Science, Misurata, for a one-week acclimatization period to the conditions of the facility.

Experimental Design

The weights of the quail ranged from 205-250g. The sublethal dose was determined by using various concentrations (1, 2, 3 and 4mg/kg) over a period of 96 hours. The value was derived using Probit analysis, resulting in a sublethal dose of 0.5mg/kg.

The birds were randomly distributed into three cages, with eight birds per cage. The groups were as follows: Group One (A) consisted of the control animals, which were not treated and received distilled water throughout the experiment. Group Two (B) included the birds that were administered diazinon at a dose of 0.5mg/kg for a duration of 21 days. After 21 days of oral administration, blood was collected from the jugular vein of all experimental animals and Placed in tubes without EDTA [13]. The samples, labeled in centrifuge tubes, were transported to the Misurata Central Laboratory using a plastic container with ice for homogenate separation. The separation was performed using a centrifuge (Hettich-420 EBA Zentrisugen) at 5000rpm for 15 minutes at -4°C. The supernatant obtained from the centrifugation was collected using a micropipette and transferred into Eppendorf tubes, which were then stored in a freezer until the subsequent steps commenced. The levels of several biochemical parameters required for this study were measured using the Cobas Integra 400 analyzer from Roche, Germany. The parameters measured included ALT, AST, and ALK.

For studying the effects of the specified diazinon dose (0.5mg/kg) on liver histology after 21 days of administration, the liver was weighed using a sensitive digital scale following the sacrifice of the animal by exsanguination. 0.5cm piece of liver was taken from all groups (control and experimental) and was immediately placed in 10% formalin for 12 to 24 hours to fixing the tissue, ensuring that the volume of formalin was double that of the sample (20 times), to adequately preserve the tissue for subsequent histological sectioning. A series of steps were then employed, beginning with processing and embedding, where the samples were subjected to a graded series of ethanol, followed by xylene, and finally infiltrated with paraffin wax. The samples were embedded in paraffin to create wax blocks. A rotary microtome was used to obtain paraffin sections, which were then stained with hematoxylin and eosin, H&E [14].

Statistical Analysis

The data obtained were analyzed using a one-way analysis of variance (ANOVA) for a completely randomized design. The Least Significant Differences (LSD) method was employed to determine significant differences at a 0.005% significance level among the means of the various treatments, utilizing the statistical software SPSS version 2010.

Results

Biochemical Study

Table 1 indicate a significant increase in ALT concentration, with an average of 37.2±2.3U/L in the diazinon-treated group, compared to an average of 11.2±0.8U/L in the control group. The current study also indicated an increase in the concentration of ALK enzyme in the diazinon-treated group (0.5±0.1U/L) compared to the control group (0.2±0.0U/L) (Table 1). The elevated ALK levels in the serum of quail were statistically significant when compared to the control group, with all values demonstrating high significance. In addition to, table 1 indicate an increase in the concentration of ALP, with a higher level observed in the diazinon-treated group (33.9±4.3U/L) compared to the control group (4.1±0.2U/L). However, the elevation in AST enzyme levels in the diazinon-treated group was not statistically significant (P>0.05).

Parameters	Means±SE (U/L)	
	Control	Treated
AST	11.2±0.8	37.2±2.3
ALK	0.2±0.0	0.5±0.1
ALP	4.1±0.2	33.9±4.3

Table 1: Average values of liver enzyme activities (U/L) in the serum of male local quail treated with a sublethal dose of diazinon (50mg/kg).

Histological Study

Microscopic examination of the liver from the control group of quail reveals various tissue components, including normal parenchymal epithelial tissue, as well as interstitial connective tissue associated with the liver. Additionally, bile ducts are observed adjacent to the central vein. In figure 1, the polygonal hepatocytes with central nuclei are evident, while figure 1, shows the proliferation and degeneration of numerous blood sinusoids within the parenchymal epithelial tissue of the liver.

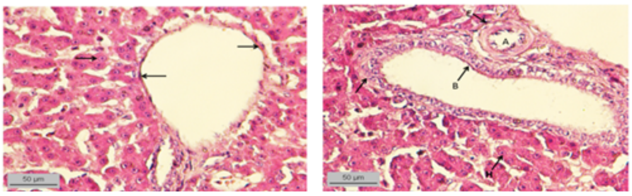

Figure 1: Normal histological structure of the liver in local quail. H&E. (A) Bile duct lumen, (B) Epithelial cells, (Arrow) Nucleus.

The current study indicates significant necrosis and degeneration in the liver tissue (Figure 2) compared to the control group. The administration of the organophosphorus insecticide (Diazinon) resulted in alterations in the shape of hepatocytes from their normal polygonal appearance observed in the control group to a swollen and amorphous form. Additionally, there was evident disruption of the connective tissue supporting the liver components, along with thrombosis in the major blood vessels and sinusoids. Microscopic examination also revealed fibrosis in the endothelial membrane of the central vein lumen (Figure 2).

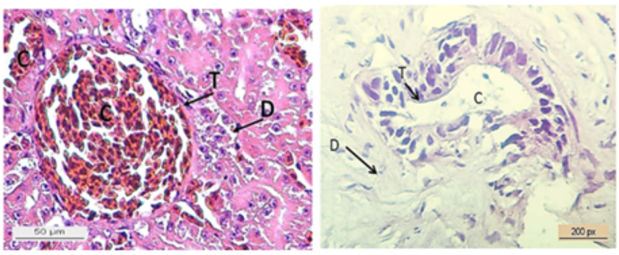

Figure 2: Histopathological changes in the kidneys of local quail exposed to a sublethal dose of diazinon (50 mg/kg). H&E. (C) Thrombosis, (T) Thickening, (D) Degeneration, (C) Lumen.

Discussion

Diazinon is an organophosphorus pesticide widely used across the globe for pest control. These compounds are characterized by their toxicity due to their persistent nature and ability to bioaccumulation in the environment. It is crucial to assess the risks associated with diazinon, taking into account the dose, exposure duration, and the specific toxicological effects. The substance poses a significant threat to both terrestrial and aquatic organisms [15].

The current study is results indicated a significant increase in the level of ALK in the serum of local quail in the group treated with a sub-lethal dose of diazinon. This finding is consistent with what Abu-Sheir [16], reported, where the ALK enzyme level increased in white rats after being administered diazinon. Additionally, the results of this study align with the findings of Karami et al. [17], who attributed the elevated ALK enzyme levels as a response of the liver to any obstruction in the bile ducts. Also observed there was a non-significant increase in the level of ALT enzyme compared to the control group. This finding is consistent with a study conducted by Abu-Sheir [16], which reported an increase in ALT enzyme levels in white rats following diazinon administration. Additionally, it aligns with research by Sarhan and Al-Sahhaf [18], on the liver and kidneys of rabbits, where the administered dose of diazinon resulted in an elevated ALT enzyme level. Furthermore, the current study's results are in agreement with the findings of Gokcimen et al. [19], who observed an increase in ALT levels in white mice exposed to diazinon for 21 days. The change in ALT levels was attributed to oxidative stress caused by free radicals, which led to the release of enzymes from the cytoplasm of damaged cells into the bloodstream [20]. Furthermore, an increase in the serum level of AST enzyme in quails treated with a sublethal dose of diazinon, with the observed elevation in AST being statistically significant ($P>0.05$). These findings are in agreement with those reported by Abu-Sheir [16], which demonstrated that diazinon leads to an increase in enzyme levels in the serum of white rats compared to the control group. Additionally, the current results are consistent with the study conducted by Gokcimen et al. [19], and also align with the findings of Munir et al. [21], regarding the white egret (*Bubulcus ibis coromandus*). The increase in AST levels is presumably due to liver cell damage and alterations in cellular membrane permeability. This disruption is explained by Fowler et al. [22] and Alyasiri et al. [23], who noted a close relationship between liver tissue damage and elevated liver enzyme levels due to oxidative stress in cellular membranes. This results in the leakage of cellular components (such as mitochondria) due to the degeneration of hepatic cells, ultimately leading to an increase in AST levels in the bloodstream.

The results of the current study showed an increase in the number of Kupffer cells and hypertrophy of the walls of the sinusoids and hepatic cords, which is in agreement with the findings of Cakici and Çömden [24]. This also aligns with the results of Alshitshat and Alhemmali [25], in their study on the effects of cypermethrin on the liver of quails. Cellular degeneration in liver tissue occurs due to reduced oxygen supply to the cells as a result of toxicity [26], along with the impact of free radicals generated by oxidation and reduction reactions occurring within the cells [25,27].

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