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Original Research Paper

An Investigation into the Hematotoxin Effects of Sublethal Diazinon Doses in Albino Rats (*Rattus norvegicus var. albinus*)

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Abstract: Diazinon, a hazardous organic insecticide, causes considerable dangers when released into the environment, notably to aquatic life and terrestrial animals. This study aims to examine the toxicological effects of diazinon on albino rats. (Rattus norvegicus var albinus), The emphasis is on deadly and sublethal concentrations. This study was carried out with albino rats kept in a controlled laboratory environment. The LD50 was determined using Probit analysis, resulting in an LD50 of 588 ppm body weight. For sub-lethal effects, rats were treated to two different dosages a dose (60 and 300 ppm) for a period of 30 days. Hematological markers were measured at intervals of 1, 15, and 30 days. The results indicate that there was a significant increase in the RBC, WBC, and HGB, in contrast with the PLT on the first day of exposure, which showed a significant decrease compared to the control group. These studies demonstrate Diazinon's toxic effect on albino rats, emphasizing its ability to damage hematological parameters at sublethal doses.

Keywords: Diazinon, Hematological markers, Sublethal effects, Albino rats

1.Introduction

Diazinon, an organophosphate pesticide, is commonly used in agriculture and urban areas to control insect pests. However, its widespread use has resulted in significant environmental contamination, causing threats to aquatic and terrestrial organisms [1]. Diazinon's toxic effects are mostly due to the suppression of acetylcholinesterase (AChE), an enzyme required for healthy nervous system function [2]. This inhibition can cause acetylcholine to accumulate, leading in nervous system overstimulation and physiological abnormalities in exposed species [3].

Non-target species in terrestrial environments, such as mammals, are especially vulnerable to Diazinon's harmful effects. Albino rats (Rattus norvegicus) are frequently utilized as model organisms in toxicological studies due to their physiological similarities to humans and sensitivity to environmental contaminants [4]. Diazinon has been linked to a variety of negative effects in rats, including hematological changes, liver dysfunction, and neurotoxicity [5]. Bioaccumulation and long-term persistence in the environment. Diazinon can disrupt hematological parameters such as white blood cell (WBC) count, platelet (PLT) count, and hemoglobin (HGB) levels, as well as induce liver damage, as evidenced by changes in enzymes such as glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) [6]. This study will look at the lethal and sub-lethal effects of Diazinon on albino rats, with an emphasis on hematological markers. By determining the sublethal concentration (LD50) and investigating the effects of sublethal concentrations.

Agricultural workers exposed to various pesticides display significantly lower hemoglobin values compared to reference groups [7].

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The PLT Environmental pollutants exert diverse effects on platelet counts and function, with both increases and decreases observed depending on the specific toxicant. Occupational exposure studies yield variable results regarding platelet parameters. While industrial workers typically show elevated platelet counts, coal miners demonstrated no significant differences in platelet concentrations compared to auxiliary workers, suggesting potential adaptation mechanisms or differential toxicant effects [8].

The MCV, Cypermethrin, a commonly used pesticide, was found to decrease MCV values in exposed subjects, suggesting potential interference with erythropoiesis or red cell maturation [9]. Cadmium exposure has been associated with reduced MCV values, with studies demonstrating that melatonin treatment can ameliorate these cadmium-induced decreases, normalizing MCV levels in experimental models [10].

2.Methodology

Samples collection

Albino Rats (Rattus norvegicus) weighed 150 ± 5g. Rats were brought in cages to the laboratory and placed in many plastic containers for 14 days to acclimate to the new circumstances (Mossa et al., 2012) the lab temperature remained at 19 ± 2 °C. During the acclimatization and testing phase, the rats were given commercial rats food. Following 14 days of acclimatization, a static acute toxicity test was performed (Juan, 2024). Al Fares Company sold diazinon with a purity of 60%, which was used to make diazinon test solutions Rats were exposed to five different concentrations of diazinon (0, 60, 300, 600, 800, 1000 ppm) to determine 1, 24, 48, 72 and 96 h LD50 value for the test rats.

The death rate was recorded after 1, 24, 48, 72 and 96 h of exposure; the Probit Analysis test was used to calculate LD50 values [11]. The sub-lethal toxicity test occurred at the end of the 96-hour acute toxicity test. Rats were treated to two sub-lethal dosage of diazinon (60 and 300 ppm) for 30 days. The hematological examinations were performed after 1, 15, and 30 days of exposure. After each exposure time, three rats from each exposure concentration, three exposure rats, were randomly selected. Rats Blood was taken by puncturing the heart, then drawn using a vacutainer needle linked to an EDTA tube (50 IU sodium heparin per ml of blood) and utilized immediately for hematological testing. The hematological parameters examined were erythrocyte count (RBC), leukocyte count (WBC), hemoglobin concentration (Hgb), platelet count (PLT), and mean corpuscular volume (MCV), which were obtained using the unified procedures for hematological assessment of rats, The auto hematology analyzer was used to obtain all the results.

3.Results and discussion

The acute toxicity assessment revealed a time-dependent increase in diazinon's lethality in albino rats (Rattus norvegicus var. albinus), as evidenced by a significant reduction in the median lethal dose (LD50) over a 96-hour exposure period. The calculated 96-hour LD50 value for albino rats was 588 mg/kg. In contrast to these findings, the sub-lethal toxicity experiment, conducted at concentrations below the determined LD50, resulted in no observable mortality across the experimental group of rats. This suggests that while acute exposure to diazinon can be lethal at specific concentrations, lower, sub-lethal doses do not induce immediate mortality in this animal model.

Red blood cells count (RBCs)

The results of RBC count show the highest value in the 300-ppm concentration (8.25 ± 0.15* ^6) on the first day and the lowest value in the 60-ppm group (3.94 ± 0.04) on the 30th day. The results also indicate a decreasing in RBC count for the control group from the 1st to the 15th day, followed by a slight increase on the 30th day.

The 60-ppm concentration shows a significant decrease in RBC count by the 30th day compared to the control group. In contrast, the 300-ppm concentration shows a decreasing trend from the 1st to the 15th day but remains higher than the control group on the 30th day

The current study disagrees with [12].

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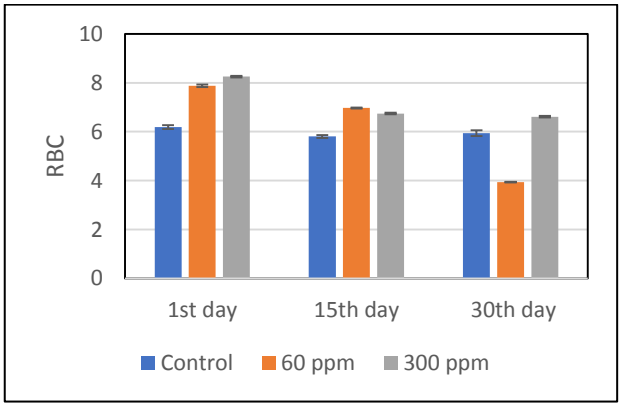

Fig 1: The RBC of Rattus norvegicus after exposure to sub-lethal concentration of diazinon

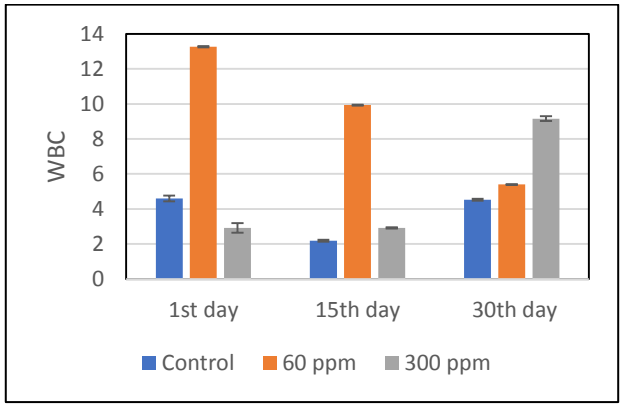

Fig 2: The WBC of Rattus norvegicus after exposure to sub-lethal concentration of diazinon

Diazinon, an organophosphate pesticide, induces oxidative stress through free radical generation, which can damage red blood cells (RBCs) and reduce their oxygen-carrying capacity [13]. This oxidative stress is linked to hemolysis, as lipid peroxidation destabilizes RBC membranes, leading to their destruction. In response to hypoxia caused by reduced oxygen availability, the kidneys release erythropoietin (EPO), a hormone that stimulates bone marrow to increase RBC production [14].

White blood cells (WBC)

The results of WBC count show the highest value in the 60-ppm group (13.26 ± 0.06* ^3) on the 1st day and the lowest value in the control group (2.19 ± 0.04* ^3) on the 15thday.

The 60-ppm concentration caused a significant increase in WBC count on the 1st day, followed by a decrease by the 15th and 30th days, though remaining higher than the control. In contrast, the 300-ppm group showed a sharp decrease in WBC count on the 1st day, remained low on the 15th day, but then increased significantly by the 30th day, surpassing both the control and 60 ppm groups.

The current study disagrees with [15].

Exposure to Diazinon can induce a pro-inflammatory immune response in rats, characterized by increased cytokine production and alterations in bone marrow activity, leading to elevated WBC levels [16]. These elevated WBC levels, particularly neutrophils, are indicative of the body's defense mechanisms being activated in response to the chemical insult. Studies have shown that Diazinon exposure can disrupt the delicate balance of the hematopoietic system, potentially leading to increased production and release of white blood cells from the bone marrow as the body attempts to counteract the inflammatory effects of the diazinon [17].

Hemoglobin estimation (Hgb)

The results of HGB levels show the highest value in the 300-ppm group (14.3 ± 0.2) on the 30th day and the lowest value in the 60-ppm group (5.9 ± 0.05) on the 30th day.

The 300-ppm concentration caused a steady increase in Hgb levels throughout the experiment, peaking on the 30th day. In contrast, the 60-ppm group showed an initial increase on the 1st and 15th days, followed by a sharp decline by the 30th day, dropping well below the control group. The current study disagrees with [18].

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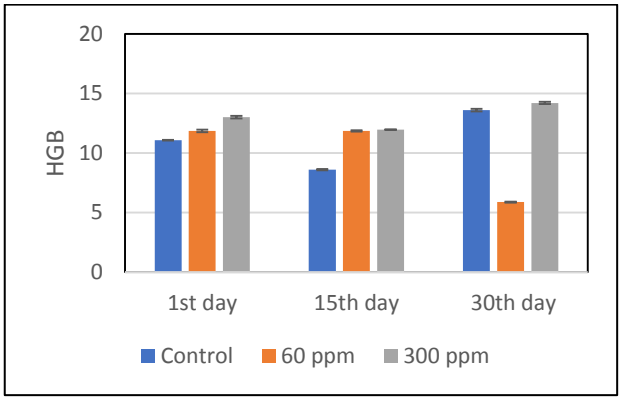

Fig 3: The Hemoglobin (Hgb) Of Rattus norvegicus after exposure to sub-lethal concentration of Diazinon

Diazinon exposure has been demonstrated to induce oxidative stress and hemolysis, consequently compromising the structural integrity and diminishing the oxygen-carrying capacity of red blood cells (RBCs). This reduction in oxygen transport efficiency precipitates tissue hypoxia, a condition promptly sensed by the kidneys. In response to this hypoxic state, renal peritubular interstitial cells augment the secretion of erythropoietin (EPO), a glycoprotein hormone crucial for erythropoiesis. EPO acts upon hematopoietic stem cells within the bone marrow, stimulating the proliferation and differentiation of erythroid progenitor cells, thereby resulting in an enhanced production of erythrocytes and hemoglobin. [2].

platelet count (PLT)

The results of PLT count show the highest value in the 300-ppm group (770 ± 3* ^3) on the 15th day and the lowest value in the 60-ppm group (96 ± 3* ^3) on the 1st day. The 300-ppm concentration caused a sharp increase in PLT count on the 1st day, peaking on the 15th day, followed by a moderate decrease by the 30th day. In contrast, the 60-ppm group showed an initial drastic drop on the 1st day, but then rose dramatically by the 15th day, before declining again by the 30th day, though remaining above control levels.

The current study disagrees with [18].

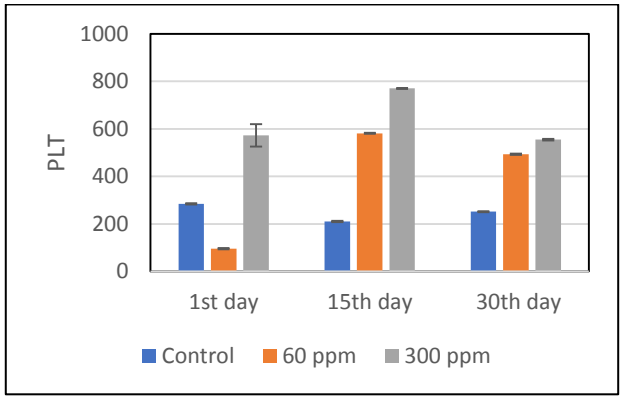

Fig 4: The Platelet (PLT) Of Rattus norvegicus after exposure to sub-lethal concentration of Diazinon

Oxidative stress caused by Diazinon increases ROS levels, including superoxide anions (O2-) and hydrogen peroxide (H2O2), which stimulate platelet production and activation nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) enzymes, particularly NOX1 and NOX2, are major sources of ROS in platelets [19].

Mean corpuscular volume (MCV)

The results of MCV (Mean Corpuscular Volume) show the highest value in the control group (54.5 ± 1.5) on the 1st day and the lowest value in the 300-ppm group (43.3 ± 0.2) on the 30th day.

The control group exhibited a gradual decrease in MCV from the 1st to the 15th day, followed by a partial recovery by the 30th day. The 60-ppm group showed an initial decrease on the 1st day, followed by a significant increase by the 15th day, before dropping again by the 30th day. In contrast, the 300-ppm group displayed a steady decline throughout the experiment, reaching its lowest point on the 30th day.

The current study disagrees with [20].

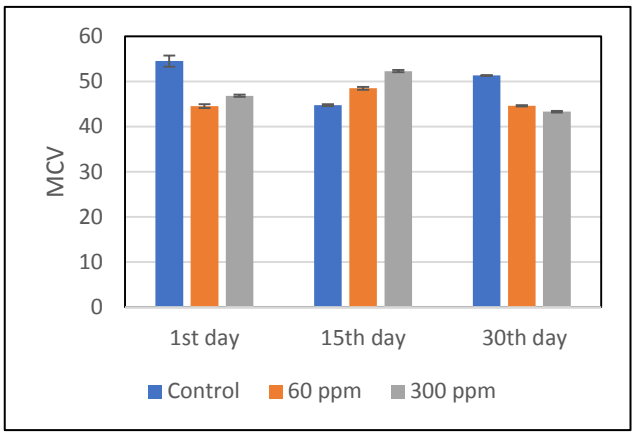

Fig 5: The mean corpuscular volume (MCV) Of Rattus norvegicus after exposure to sub-lethal concentration of diazinon

The decrease in mean corpuscular volume (MCV) observed in Diazinon-exposed groups indicates the development of microcytic anemia, characterized by red blood cells that are smaller than normal. This reduction in cell size typically reflects impaired hemoglobin synthesis, as hemoglobin is essential for oxygen transport and influences red blood cell size, the decrease in MCV in Diazinon-exposed groups is a sign of microcytic anemia, reflecting smaller red blood cells due to impaired hemoglobin synthesis, likely caused by Diazinon toxicity affecting erythropoiesis and iron metabolism through oxidative stress mechanisms [18,21].

Conclusion

In conclusion Diazinon has a harmful effect on the blood parameters after exposure to sub-lethal concentration, which was vividly clear on the results.

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Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017).

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