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Toxicological and Oxidative Stress Effects of Imidacloprid on Hematological and Biochemical Parameters in Rats

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Abstract: Neonicotinoid pesticides are considered a good alternative to organophosphate pesticides, although there are reports of adverse health effects in rats. This study aimed to determine the sub-chronic effects on hematological and biochemical markers in male rats 28 days after oral administration of imidacloprid at concentrations of 5, 10, and 20 mg/kg body weight. Results: Hemoglobin levels, red blood cell count, and platelet count were significantly decreased in all treatment groups after exposure, while white blood cell count was increased in all treated rats compared to the control group. In addition, 28 days after exposure, oxidative stress markers showed a statistically significant increase (P≤0.05) in malondialdehyde (MDA) levels and a decrease in reduced glutathione (GSH) levels and catalase (CAT) activity compared to the control group.

Keywords: Pesticide, Neonicotinoid, Sub Chronic, Imidacloprid, Oxidative Stress.

RESEARCH PAPER

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1-INTRODUCTION

One of the most popular insecticides in the world is imidacloprid (IMI). It is highly effective at controlling agricultural pests because it is a member of the neonicotinoid group, which targets acetylcholine nicotinic receptors in the insect nervous system. However, the extensive usage of this substance has sparked growing worries about its possible harmful effects on non-target creatures, such as mammals, as a result of repeated or prolonged exposure(Zouaoui & Rouabhi, 2024).

A growing body of research indicates that imidacloprid may primarily cause dysfunction of the central nervous system through a multi-stage process, most notably oxidative stress, which contributes to cellular cable carving by increasing reactive oxygen species (ROS) production and depleting antioxidants. Reduced glutathione (GSH), elevated peroxide products such malondialdehyde (MDA), and changes in antinutrient enzymes like oxidase deoxyribonuclease (SOD), catalase (CAT), and glutathione peroxidase (GPx) have all been connected to this hyperoxidative dysfunction(AL-Janabi & Abdulhay, 2025).

Other than oxidative stress, mitochondrial dysfunction may lead to decreased energy generation, a rise in membrane permeability, and activation of cell death pathways, which are all significant contributors to neurotoxicity. Lysosomes, which are essential in maintaining cellular homeostasis, can also be damaged due to exposure to toxins. This may cause loss of stability, pH imbalances and hydrolytic leakage of enzymes, which further aggravates cell damage (Gergs *et al*., 2021).

The neurotoxicity of imidacloprid has been reported, and there is little understanding of the relationship between oxidative stress, mitochondrial dysfunction, and lysosomal instability and neurotoxicity, particularly in chronic exposure. To determine the cellular and molecular changes in neurotoxicity of chronic imidacloprid exposure in Wistar rats, markers of oxidative stress, assessment of mitochond (Abd-Elhakim *et al*., 2018)

2-MATERIAL & METHODS

Experimental Design: Twenty-four rats weighing between 180 and 220 grams were employed from the animal house at Al-Qadisiyah University's College of Science. The rats were about two and a half months old when they achieved maturity. The animals were kept in cages with natural humidity levels (45–60%) and temperatures (22–26°C). The rats were split into four equal groups, each with six rats, after ten days of acclimation. The control group was assigned to the

first group. For 28 days, the second and third groups received 10–20 mg/kg body weight of imidacloprid, while the first group received 5 mg/kg body weight of imidacloprid.

2-1 Gathering and Examining Samples

All animals underwent cardiac aspiration to obtain fresh blood samples following a 28-day course of medication.After evaluating the complete blood count (CBC), these samples were taken. For biochemical examination, the serum samples were put in tubes at -20°C. serum levels of malondialdehyde (MDA), reduced glutathione (GSH), and catalase (CAT) activity were measured using specialized colorimetric test kits from the biotechnology company Sigma-Aldrich and an ELISA kit according to (Fossati & Prencipe, 1982)and (Buege & Aust, 1978) as well as commercially available colorimetric assays.Measurements were made of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), The measurement was based on the methods mentioned in(Kipriyanov & Little, 1997), (Allain *et al*., 1974) and (Friedewald *et al*., 1972).

3-RESULTS

3-1 Hematological Parameters

The findings showed that RBC, hemoglobin, hematocrit, and platelet count decreased in a dose-dependent manner, whereas WBC count significantly rose as imidacloprid exposure levels increased ($p < 0.05$) as shown in Table (1), Rats given different doses of imidacloprid for 28 days did not exhibit any symptoms of death. Imidacloprid has a dose-dependent effect on hematological parameters, according to the data, and the degree of alterations increases as doses increase from 5 to 20 mg/kg. Red blood cell (RBC) counts varied significantly between the lowest and highest doses, falling from 7.92 in the control group to 4.98 at the highest dose. This confirms that the slow decline is dose-related and shows the onset of anemia brought on by bone marrow suppression or red blood cell destruction, The red blood cell count result was similar to those reported by (A.khafagy, 2022), who reported a significant decrease in red blood cell count after imidacloprid treatment of mice.

The level of hemoglobin decreased in the control group to 14.35 g/dL, and in the group taking the highest dose, to 9.14 g/dl. Oxidative anemia being directly associated with decrease in red blood cells implies that the ability of the blood to carry oxygen is decreased. These findings are completely in line with what was mentioned in (Chakroun *et al*., 2016). This study showed a significant decrease in hemoglobin levels and red blood cell count as a result of exposure to the pesticide acetamiprid, which is from the same neonicotinoid group, where the decrease in hemoglobin was explained by an increase in the amount of oxidized hemoglobin. Moreover, the reduction in the hemoglobin level, with a corresponding reduction in red blood cell count could be attributed to the destruction of red blood cell membranes by pesticides resulting in hemolysis or disproportionate distribution of blood iron.

As for hematocrit, it showed a decrease from 43.12% in the control group to 28.57% at the highest dose. This may be due to the fact that hematocrit represents the percentage of red blood cell volume in the blood, and its decrease confirms true anemia, not apparent anemia resulting from a decrease in the number and size of red blood cells (Tonietto *et al.*, 2022).

Conversely, the white blood cell count increased from 7.85 in the control group to 12.76 × 10^3/µL at the highest dose, with a significant increase from the lowest dose to the highest. This indicates an inflammatory response or activation of the immune system, which usually occurs as a result of oxidative stress and tissue damage, however, the effect of the pesticide on platelets decreased from 618 to 378 ×10^3/µL. It could be because platelet production in the bone marrow will have been inhibited or that the inflammation will have consumed more of it (Suwannarin *et al*., 2021). These hematological changes can be explained by the effects of oxidative stress. It is thought that exposure to imidacloprid will have considerably increased the malondialdehyde (MDA) levels which is an essential indicator of lipid peroxidation and reduced the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD). This oxidative imbalance directly damages the unsaturated fatty acid-rich membranes of red blood cells and accelerates the formation of reactive oxygen species (ROS) and predisposes the cells to hemolysis. This can be attributed to the observed fall in RBC, Hb, and HCT. (Obeagu *et al*., 2024).

Table 1: Effect of Imidacloprid on hematological parameters of male rats for 28 days

Groups	RBC (×10^6/µL)	WBC (×10^3/µL)	Hb (g/dL)	HCT (%)	PLT (×10^3/µL)
Control	7.92 ± 0.45^a	7.85 ± 0.52^d	14.35 ± 0.61^a	43.12 ± 1.28^a	618.27 ± 26.4^a
IMI-5 mg/kg	6.83 ± 0.39^b	9.21 ± 0.63^c	12.48 ± 0.57^b	38.76 ± 1.34^b	542.18 ± 23.7^b
IMI-10 mg/kg	5.94 ± 0.36^c	10.87 ± 0.69^b	10.86 ± 0.52^c	33.41 ± 1.26^c	461.55 ± 21.9^c
IMI-20 mg/kg	4.98 ± 0.31^d	12.76 ± 0.74^a	9.14 ± 0.48^d	28.57 ± 1.19^d	378.64 ± 20.8^d
LSD	0.46	0.78	0.66	1.53	28

3-2 Indicators of Oxidative Stress

Findings of the present research indicated that long-term treatment with imidacloprid causes oxidative stress in the treated mice relative to the control group as indicated by the variation in the levels of MDA, GSH and CAT. Imidacloprid drastically changes the levels of MDA, CAT and GSH in treated mice who were significantly elevated compared to the control group (Abdel-Halim & Osman, 2020). The CAT and GSH level in all the treatment groups after 28 days were significantly lower ($p<0.05$) than the reported mean. CAT levels were (3 ± 38, 2 ± 30, and 45 ± 4, respectively), while GSH levels were (0.2 ± 3.5, 0.3 ± 5.0, and 0.4 ± 6.5, respectively) compared to the control group (Tonietto *et al*., 2022).

Moreover, Table 2 revealed a significant difference ($p<0.05$) in the level of MDA in the blood of imidacloprid-treated mice after 28 days. This varied between $3.5 + 0.3$ with the low dose of the pesticide and $7.0 + 0.5$ with high dose and it directly depended on the dose concentration, which was $2.0 + 0.2$ in the control group. High levels of metabolites of reactive oxygen species, especially hydroxyl radicals, that regulate the antioxidant defense system could be the cause of the higher level of malondialdehyde (MDA) in the imidacloprid and nano-imidacloprid-treated rats (Liu *et al*., 2025).

This study shows that long-term exposure to imidacloprid causes a variety of hematological, oxidative, and histopathological alterations that are suggestive of systemic toxicity. The drastic reduction in hemoglobin, hematocrit and red blood cell count in the treated groups indicates anemia that might have been precipitated by a decreased production of red blood cells or increased destruction of red blood cells. The dramatic increase of the malondialdehyde (MDA) and absence of essential antioxidants such as glutathione (GSH) and catalase (CAT) suggest that these are hematological changes that are closely connected to the oxidative stress (Abd-Elhakim *et al*., 2018). Since red blood cells contain a lot of polyunsaturated fatty acids, and are often exposed to oxygen, high MDA concentrations are an indicator of increased lipid peroxidation in cell membranes. Oxidative damage leads to membrane injury and hemolysis, and reduced survival of red blood cells. At the same time, reduced concentrations of catalase (CAT) and glutathione (GSH) are indicative of a stressed antioxidant defense system, which augments oxidative injury. The findings are consistent with previous studies that indicate that imidacloprid enhances the production of reactive oxygen species (ROS), which oxidatively damages tissues and blood cells (Suwannarin *et al*., 2021). As a result, the increased white blood cell count could be an adaptive inflammatory reaction to oxidative stress and tissue damage. More reactive oxygen species are produced by activated immune cells, which raises the oxidative burden and starts a vicious cycle of inflammation and cell damage. Also, the severe decrease of platelets (thrombocytopenia) makes it possible that either peripheral destruction or bone marrow suppression took place due to oxidative processes that influence neutrophil and platelet survival (Kapoor *et al*., 2010).

It is interesting to note that histopathology findings where structural damage is often found in vital organs such as liver, kidneys and spleen following imidacloprid use significantly contribute to these biochemical and hematological deficiencies. Cell necrosis, tissue inflammation, inflammatory cell infiltration, and vascular compression are some examples of these lesions. These tissue-scale changes can be mechanistically justified by the oxidative stress damage of cellular macromolecules, including proteins, lipids and DNA. Furthermore, the specified systemic alterations in hematology are due to the dysfunction of organ activity produced by the alterations in the cell structure (Zouaoui & Rouabhi, 2024).

When these outcomes are considered, the combination of hematological indicators and biomarkers of oxidative stress with histological evidence will allow obtaining a comprehensive understanding of imidacloprid toxicity. The results indicate that oxidative stress is one of the most important mechanisms that bridges the relationship between cell damage and functional changes in tissue integrity and hematological parameters, furthermore, the dose-dependent nature of the effects highlights the potential health risks related to long-term exposure and supports the toxicological applicability of imidacloprid.

Table 2: On the oxidative stress parameters of male rats after 28 days of imidacloprid effect

المجموعة	MDA (nmol/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)
Control	2.0 ± 0.2	55 ± 3	8.0 ± 0.5
Imidacloprid-Low (5 mg/kg)	3.5 ± 0.3 ↑	45 ± 4 ↓	6.5 ± 0.4 ↓
Imidacloprid-Medium (10 mg/kg)	5.0 ± 0.4 ↑	38 ± 3 ↓	5.0 ± 0.3 ↓
Imidacloprid-High (20 mg/kg)	7.0 ± 0.5 ↑	30 ± 2 ↓	3.5 ± 0.2 ↓

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