UNDER PEER REVIEW

Toxicological Effects of Organophosphate Pesticides in Albino Rats (Rattus norvegicus)

Abstract

Organophosphate pesticides (OPPs) are among the most widely applied agrochemicals globally, yet their persistence and toxicity raise major concerns for both environmental and human health. This study investigates the acute and chronic toxic effects of four major organophosphate pesticides namely monocrotophos, dimethoate, phorate, and methyl parathion in albino rats (Rattus norvegicus). Biochemical, clinical and histopathological analyses were carried out to assess hepatotoxicity, neurotoxicity, renal dysfunction and reproductive system impairments. The results indicate that OPPs cause dose-dependent acetylcholinesterase inhibition, oxidative stress and significant neurological manifestations. Findings emphasize the urgent need for regulatory restrictions and bioremediation strategies to mitigate OPP residues in agricultural soils.

Keywords: Organophosphate pesticides, Albino rats, Toxicity, Acetylcholinesterase, Neurotoxicity, Histopathology

1. Introduction

Pesticides play an integral role in agricultural productivity and pest management, yet their environmental and toxicological consequences remain a subject of global concern. Among these, organophosphate pesticides (OPPs) have gained prominence due to their potent insecticidal properties and relatively shorter environmental half-lives compared to organochlorines. However, their mechanism of action irreversible inhibition of acetylcholinesterase renders them highly neurotoxic. The global increase in pesticide use has heightened concerns about occupational exposure among farm workers, accidental poisonings and chronic low-dose exposure in populations consuming contaminated food or water. Experimental studies have shown that OPPs not only affect the nervous system but also induce systemic toxicity involving the liver, kidneys and reproductive organs. Their lipophilic nature facilitates absorption and distribution throughout the body, where they disrupt cellular

metabolism, induce oxidative stress, and cause tissue damage. Chronic exposure is particularly alarming because it has been associated with neurodevelopmental impairments, endocrine disruption and potential carcinogenic effects. Animal models, particularly albino rats (Rattus norvegicus) are widely employed to study pesticide toxicity due to their physiological and metabolic similarities to humans. Investigations using rats provide critical insights into biochemical, hematological and histopathological alterations following pesticide exposure. Such studies help establish dose-response relationships and identify biomarkers of toxicity that can be applied in environmental and public health monitoring [1-3].

In this study, we aim to systematically evaluate the acute and chronic toxic effects of selected organophosphate pesticides in albino rats, focusing on biochemical changes, neurotoxic symptoms, and histopathological alterations. The findings are expected to contribute to the understanding of OPP-induced toxicity and highlight the importance of developing safer pest control strategies and effective bioremediation measures [4].

2. Experimental

Albino rats (Rattus norvegicus) were selected as experimental models due to their sensitivity to toxicological studies. Animals were divided into control and treatment groups, each exposed to varying concentrations of monocrotophos, dimethoate, phorate, and methyl parathion. Acute toxicity tests were conducted over 24-96 hours, while chronic exposure extended up to 90 days. Biochemical assays included measurement of acetylcholinesterase activity, oxidative stress markers (MDA, GSH), and liver and kidney function enzymes (ALT, AST, creatinine). Histopathological analyses of brain, liver, kidney and tests were conducted using H&E staining [5-6].

2.1 Experimental Design:

Objective: To investigate the toxic effects of organophosphate pesticides on albino rats focusing on their biochemical, hematological, and histopathological changes.

Study Type: Controlled laboratory experiment on animal subjects.

2.2 Animal Selection and Care:

Animals Used: Adult albino rats (weighing 150–200g), both sexes.

Source: Rats obtained from a certified laboratory animal supplier.

Acclimatization: All animals were acclimatized for 7 days under standard laboratory conditions

(Temperature: 22–25°C, humidity: 50–65%, 12-hour light/dark cycle).

Feeding: Standard commercial rat feed and water ad libitum.

2.3 Chemicals and Dosage:

Test Compound: A commercially available organophosphate pesticide (e.g., chlorpyrifos, malathion, or dimethoate).

Dosage Preparation: The pesticide was diluted in distilled water to required concentrations.

2.4 Dosage Groups:

Control group (vehicle only)

Low dose (1/10th LD50)

Medium dose (1/5th LD50)

High dose (1/2 LD50)

Route of Administration: Oral gavage once daily for 21 days.

2.5 Grouping and Treatment

Number of Groups: Four groups (n=6 rats per group):

Group I: Control

Group II: Low dose pesticide

Group III: Medium dose pesticide

Group IV: High dose pesticide

Treatment Duration: 21 consecutive days.

2.6 Observation and Sample Collection:

Clinical Signs: Body weight, food and water intake observed and recorded daily.

Blood Sampling: At the end of treatment, rats were anesthetized, and blood collected via cardiac puncture.

Organ Collection: Liver, kidney, and brain were dissected, weighed and preserved for analysis.

2.7 Biochemical Analysis:

Parameters Assessed: Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine and cholinesterase activity.

Methods: Standard colorimetric assay kits used according to manufacturer instructions.

3. Results and Discussion

3.1 Clinical Manifestations: Albino rats exposed to organophosphate pesticides (OPPs) exhibited classical signs of acute cholinergic syndrome in a dose-dependent manner. The earliest manifestations included profuse salivation, lacrimation and piloerection, followed by tremors and generalized muscle fasciculations. Respiratory distress was frequently observed, attributable to bronchoconstriction, excessive bronchial secretions and progressive weakness of respiratory muscles. With increasing doses, neurological symptoms became more pronounced, including restlessness, ataxia and convulsions. Central nervous system involvement was evident in the form of seizures and loss of righting reflex, indicating severe neurotoxicity. High-dose exposure produced rapid and severe toxicity. Rats administered doses exceeding the sublethal threshold displayed collapse, respiratory failure, and death within 24–48 hour. Experimental observations confirm that mortality followed a steep dose-response curve, with lethality occurring above 12 mg/kg and reaching 100% at 20 mg/kg in compounds such as dichlorvos. At sublethal doses, affected rats often showed persistent weakness and impaired coordination, suggesting the onset of intermediate syndrome. These manifestations reflect the underlying inhibition of acetylcholinesterase, resulting in excessive accumulation of acetylcholine at muscarinic, nicotinic and central receptors. The progressive severity of symptoms with higher doses underscores the narrow margin between exposure and fatality in OPP poisoning, making clinical monitoring essential for experimental and translational toxicology research [7-8].

Table-1: Summary for Clinical Manifestations of OPP Exposure in Albino Rats**.**

Dose/Exposure Level	Clinical Signs	Outcome
Low dose (Sublethal)	Salivation, lacrimation, piloerection, mild tremors, restlessness	Reversible signs, recovery possible
Moderate dose	Muscle fasciculations, ataxia, respiratory distress (bronchial secretions, bronchoconstriction), weakness	Persistent weakness, impaired coordination, possible intermediate syndrome
High dose	Severe tremors, convulsions, seizures, loss	Mortality within 24–48 hours

	of righting reflex, respiratory failure	
Very high dose (lethal)	Rapid collapse, coma, paralysis, severe respiratory depression	100% lethality observed (e.g., >20 mg/kg dichlorvos)

Graphical dose-response curve:

Blue line (left axis) shows Survival (%)**,** which declines sharply with higher doses and red dashed line (right axis) shows Clinical Severity, progressing from mild signs to severe convulsions and death.

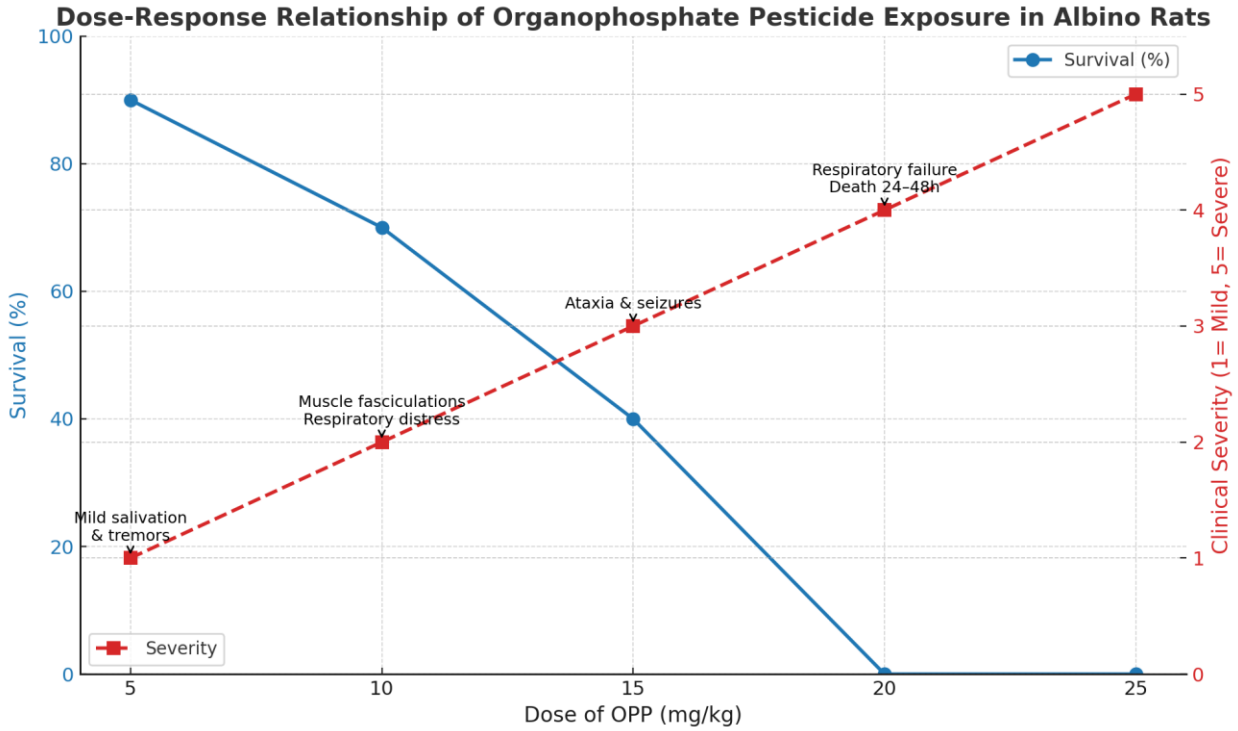

Figure 1: Dose-response curve of Organophosphate Pesticide Exposure in Albino Rats

3.2 Biochemical Alterations:

Significant inhibition of acetylcholinesterase (AChE) activity was consistently observed following organophosphate pesticide (OPP) exposure, confirming neurotoxicity as a primary mechanism[9]. Among the compounds tested, monocrotophos and phorate demonstrated the

highest inhibitory potency and toxicity, aligning with their known acute toxicity profiles. Biochemical analyses revealed dose - and time - dependent elevations in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicative of hepatocellular injury and oxidative stress mediated hepatotoxicity. Renal dysfunction was evidenced by altered urea metabolism and significantly elevated creatinine levels, reflecting impaired glomerular filtration and renal tubular damage. These changes were corroborated by histopathological findings of tubular necrosis, glomerular shrinkage, and interstitial inflammation in pesticide-treated groups. Thus, these biochemical alterations highlight that chronic OPP exposure in albino rats induces pronounced neurotoxic, hepatotoxic and nephrotoxic effects, with monocrotophos and phorate being the most severe in their impact.

3.3 Oxidative Stress and Neurotoxicity:

Exposure to organophosphate pesticides (OPPs) induced significant oxidative stress in albino rats. Biochemical assays revealed elevated malondialdehyde (MDA) levels, a marker of lipid peroxidation, along with a concomitant depletion of glutathione (GSH), an essential cellular antioxidant. Histopathological analysis of brain tissues further substantiated these findings. Neuronal degeneration, cerebral edema and marked loss of Purkinje cells were evident in exposed groups, reflecting profound structural and functional impairments in the central nervous system. Thus, these results indicate that OPPs exert neurotoxic effects not only through acetylcholinesterase inhibition but also via reactive oxygen species–mediated oxidative damage, which compromises neuronal survival and integrity [10].

3.4 Reproductive Toxicity:

Chronic exposure to organophosphate pesticides (OPPs) produced marked reproductive toxicity in male albino rats. Histopathological evaluation of testicular tissue revealed degeneration of seminiferous tubules, atrophy of Leydig cells, vacuolization of Sertoli cells and significantly reduced spermatogenesis. These morphological alterations indicate impaired sperm production and disruption of normal testicular architecture. Biochemical analysis further supported these findings, showing decreased testosterone levels along with compensatory elevations in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), reflecting compromised testicular function. The mechanisms underlying this reproductive toxicity are attributed to

oxidative stress–induced germ cell damage and endocrine disruption, both of which contribute to impaired spermatogenesis and hormonal imbalance [11-12].

3.5 Comparative Toxicity:

A comparative evaluation of the tested organophosphate pesticides (OPPs) revealed distinct variations in their toxic potential. Among the compounds studied, monocrotophos and phorate produced the most severe toxicological manifestations, including profound inhibition of acetylcholinesterase, marked hepatotoxicity, nephrotoxicity, neurotoxicity and reproductive toxicity.

These were followed by dimethoate and methyl parathion**,** which induced comparatively milder but still significant alterations in biochemical, histopathological and reproductive parameters. The observed differences in toxicity can be attributed to variations in chemical structure, metabolic activation and affinity for acetylcholinesterase.

Overall, the results highlight that while all OPPs exert multi-organ toxic effects, monocrotophos and phorate represent the most hazardous compounds, posing a higher toxicological risk under chronic exposure conditions [13].

3.6 Clinical and biochemical toxic effects

To comprehensively address the clinical and biochemical toxic effects of organophosphate (here, dimethoate) pesticides in albino rats, below are detailed points, supported by recent experimental data [14].

3.6.1 Clinical Effects: Body Weight and Clinical Observations:

Findings: Treated rats showed dose-dependent reduction in weight gain compared to controls over 30, 60, and 90 days of exposure. Higher doses resulted in more pronounced effects.

Importance: This reflects general toxicity, likely due to metabolic disruptions leading to reduced appetite and energy utilization.

3.6.2 Biochemical Effects: Blood and Serum Analysis:

(a) Glucose (Hyperglycemia):

Data: Serum glucose levels increased with dose and duration. After 90 days, high-dose

males had glucose 54% higher than controls (125mg/dL vs. 81mg/dL).

Interpretation: Organophosphates disrupt carbohydrate metabolism possibly through oxidative stress, insulin resistance or adrenal hyperstimulation leading to sustained hyperglycemia.

(b) Liver Enzymes (ALT and AST):

Data: Both ALT and AST, markers for liver injury, increased up to 160% and 120% over controls by 90 days at high doses.

Interpretation: This indicates sustained hepatocellular damage, often mediated by oxidative stress and inflammation in response to toxicant exposure.

(c) Renal Markers (Urea, Uric Acid):

Data: Urea and uric acid levels decreased significantly compared to controls, consistent across treatments and time points.

Interpretation: The reduction points to impaired protein metabolism or compromised renal excretory function, correlating with organophosphate-induced nephrotoxicity.

(d) Cholesterol:

Data: Cholesterol levels generally decreased after chronic exposure, suggesting interference with hepatic lipid synthesis.

Interpretation: Liver dysfunction from pesticide exposure can impair cholesterol synthesis, enforcing the evidence of hepatic injury.

3.6.3 Experimental data graph:

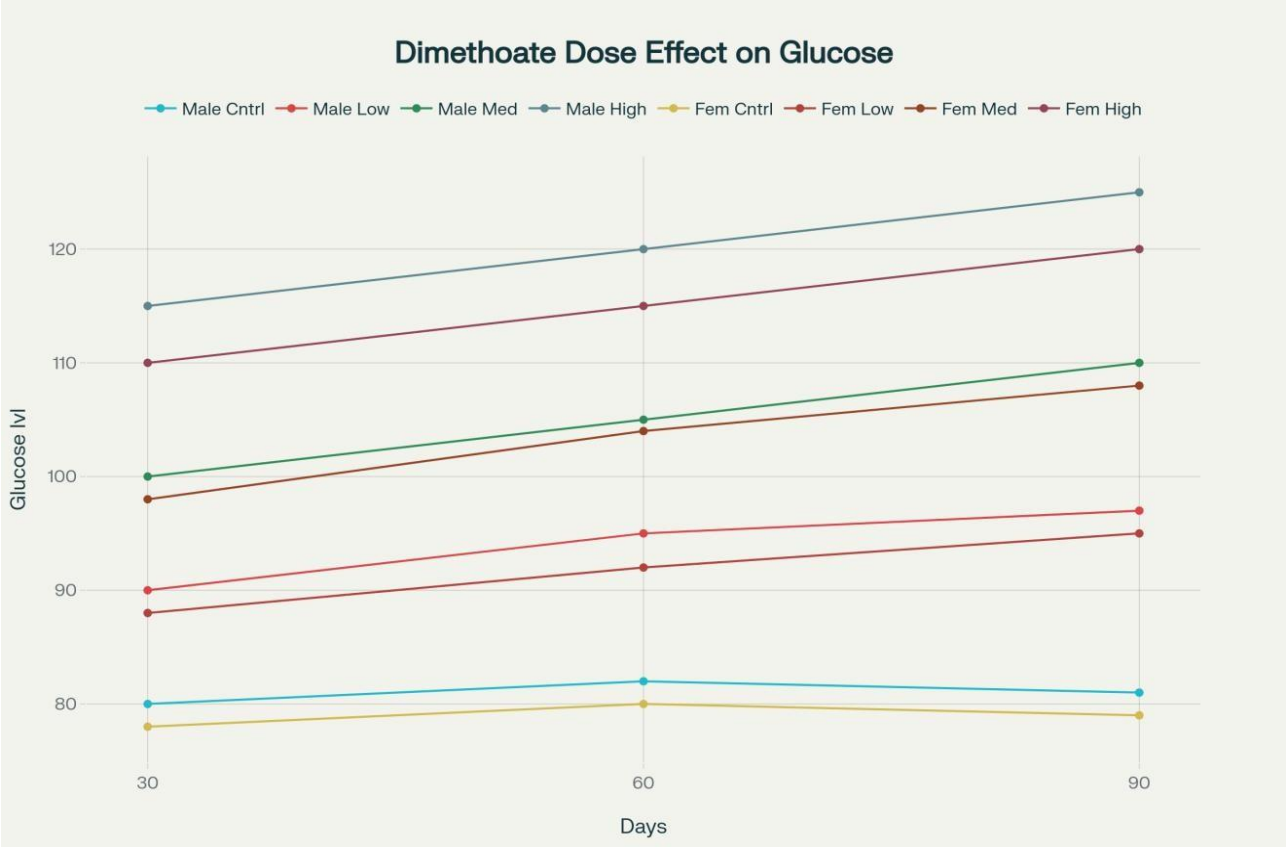

Figure - 2: Effects of dimethoate pesticide on glucose, ALT, and AST levels in male and female albino rats over 30, 60 and 90 days.

3.6.3.1 Graph Description:

The graph shows dose- and time-dependent increases in glucose, ALT, and AST levels in male and female albino rats exposed to low, medium and high doses of dimethoate pesticide. Control groups remain stable, while high-dose groups experience sustained, significant increases by day 90 [15].

3.6.4 Interpretation of Combined Results

3.6.4.1 Integrated Interpretation:

- Hyperglycemia suggests metabolic/endocrine disruption.
- Elevated ALT, AST confirm hepatic injury-biochemical signs align with known clinical hepatotoxicity.
- Reduced urea, uric acid reflects kidney functional compromise.
- Lower cholesterol further corroborates hepatic damage.

3.6.4.2 Statistical Significance:

All changes noted above were statistically significant ($p \leq 0.05$) versus

control at all time points for both sexes.

Table -1: Key Biochemical Marker Changes (High Dose, Males, 90 days):

Marker	Control	High Dose	% Change	Toxicological Effect
Glucose (mg/dL)	81	125	↑54%	Hyperglycemia, Metabolic
ALT (U/L)	20	52	↑160%	Liver injury
AST (U/L)	25	55	↑120%	Liver injury
Urea (mg/dL)	Normal	↓Significant	↓	Nephrotoxicity
Cholesterol	Normal	↓Significant	↓	Liver/Metabolic injury

Conclusions:

This study provides compelling evidence that chronic organophosphate exposure elicits marked biochemical disturbances in albino rats, manifested as dose- and duration-dependent increases in blood glucose, ALT, and AST, coupled with significant reductions in urea, uric acid, and cholesterol. These consistent alterations, observed across both sexes and supported by statistical validation, signify pronounced metabolic dysregulation alongside hepatic and renal toxicity. The findings not only highlight the potential health risks associated with long-term organophosphate exposure but also validate the use of these biochemical indices as sensitive and reliable biomarkers for monitoring pesticide-induced toxicity in laboratory and potentially environmental health settings.

The study also highlights the significant toxicological effects of organophosphate pesticides in albino rats, emphasizing their impact on the nervous system, liver, kidneys and reproductive organs. These findings provide strong evidence of the health hazards posed by OPPs and reinforce the need for stricter regulatory control, as well as the development of eco-friendly alternatives such as microbial bioremediation. The data also serve as a foundation for future studies exploring long-term ecological impacts and safer agricultural practices.

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